VOLUMEN 8 JULIO, 1960 FASCICULO 1

UNIVERSIDAD DE COSTA RICA

REVISTA
DE
BIOLOGIA TROPICAL



SAN JOSE COSTA RICA

Los artículos que se deseen someter para su publicación deberán ser enviados a: Dirección de "REVISTA DE BIOLOGIA TROPICAL", Universidad de Costa Rica, Ciudad Universitaria, Costa Rica, A. C. La correspondencia relativa a canje y suscriciones deberá dirigirse a: Biblioteca de la Universidad de Costa Rica, Ciudad Universitaria, Costa Rica, A. C.





REVISTA

DE

BIOLOGIA TROPICAL

UNIVERSIDAD DE COSTA RICA

VOLUMEN 8 JULIO, 1960 FASCICULO 1

SUMARIO

GILMOUR, E. F.—On the Neotropical Acanthocinini, X. (Coleoptera, Cerambycidae, Lamiinae). Some new species of *Urgleptes* Dillon 1

ZELEDÓN, R.—Comparative physiological studies on four species of hemoflagellates in culture. III. Effect of the Krebs' cycle intermediates on the respiration 25

VARGAS, L.—The subgenera of *Culicoides* of the Americas (Diptera, Ceratopogonidae) 35

ARROYO, G., y R. BOLAÑOS.—*Salmonella* en bovinos adultos, aparentemente sanos, destinados al consumo 49

JIMÉNEZ-QUIRÓS, O., y G. ARROYO.—Helmintos de la República de Costa Rica. XIV. Redescripción de *Lutztrema obliquum* Travassos, 1941, y descripción de *Zonorchis macroovaricus* n. sp. 53

JIMÉNEZ-QUIRÓS, O., y G. ARROYO.—Helmintos de la República de Costa Rica. XV. Nematoda 3. Presencia de *Litomosoides peñai* n. sp. en *Hemiderma perspicillatum aztecum* (Saussure, 1869) 63

RODRÍGUEZ, R. L.—Un híbrido natural de *Hydrocotyle* (Umbelliferae) 69

WILLE, A.—The phylogeny and relationships between the insect orders 93

SÁENZ, G.—Inoculación experimental de *Sporotrichum schenckii* en embrión de pollo 123

ZELEDÓN, R., W. HIDALGO y HILDA X. DE HIDALGO.—Intradermo-reacción de Montenegro con antígeno de *Strigomonas oncopelti*. Nota previa 145



Rev. Biol. Trop., 8 (1): 1-23, 1960

On the Neotropical Acanthocinini, X. (Coleoptera, Cerambycidae, Lamiinae).
Some new species of **Urgleptes** Dillon

by

E. Forrest Gilmour*

(Received for publication January 11, 1960)

In this paper, which forms a further contribution to my studies on the Neotropical Acanthocinini, are described eight new species of the genus *Urgleptes* Dillon from Guatemala, based on material from the American Museum of Natural History, New York.

These small Lamiids appear to occur in their greatest profusion of species in Central America, with minor extensions northwards into Mexico and southwards into Brazil. Probably almost 50 species are known in the genus at present, including those described herein, and others which I have described and await publication.

The genus *Urgleptes* Dillon was split off from *Lepturges* Bates in 1956 (1) and I myself have transferred 32 other species from *Lepturges* to *Urgleptes* in 1959 (2).

It is likely that other species await discovery if many parts of Central America, and elsewhere, could be more systematically examined from a field collecting point of view.

Urgleptes pareuprepes sp. nov.

Figure 1.

MALE: Fairly dark ferruginous, with greyish pubescence and dark brown maculae. Head dark brown with thin pubescence, a little greyish in parts.

---

* Director, Museum and Art Gallery, Doncaster, England.

Pronotal disc with four dark vittae:—two median, not quite reaching the base, strongly widening at the apex and almost uniting medially; a curved vitta on each side above the lateral tubercles, not reaching the apex. Scutellum thinly greyish medially, brown laterally. Elytra with the suture narrowly dark brown, without other dark markings reaching it; each elytron dark maculate as follows: the humerus, extending sinuately towards the suture and narrowing to about the basal third, and with a narrow projection to the base alongside the scutellum; in a subparallel, somewhat oblique, line, between about basal third and middle, three rather elongate-oval maculae, the subsutural median, the lateral largest at the edge of the disc extending to about the basal third, and more narrowly uniting with a dark brown macula, which extends, somewhat sinuately, posteriorly, to join a broad sinuate fascia at about apical two-fifths, which narrows to the suture, but does not quite reach it; on about the apical fifth a transverse bilobed macula which unites laterally with a projection from the postmedian fascia; at the apex an oval macula. Ventral surface ferruginous, greyish pubescent, much thinner medially. Antennae ferruginous, the apices of the segments blackish; very thinly greyish pubescent. Legs ferruginous, the femora extensively darkened; tibiae with about their apical half, and the tarsi, dark ferruginous; thinly fulvous-grey pubescent.

Small; elongate-ovate; subdepressed. Antennae elongate and slender, (segments missing after sixth segment, but exceeding the elytral apex from about the middle of the fifth segment, and probably well over twice as long as the body); sparsely, but distinctly setose below from the scape preapically to the fourth segment; scape a little and regularly swollen to the apex, elongate, extending slightly past the pronotal base; third segment very slightly longer than the scape; fourth segment almost one and a quarter times as long as the third; fifth and sixth about equal, each slightly shorter than the fourth and slightly longer than the third; (remaining segments missing); segments finely and fairly closely punctured. Antennal tubercles fairly strongly swollen, moderately raised, very broadly V-shaped between; feebly and broadly foveate medially behind the tubercles. Frons large, about quadrate, very feebly narrowed above between the eyes, fairly strongly convex, with a very fine median longitudinal line; head very finely and closely alutaceously punctured. Eyes fairly small; lower lobe rather small, rotundate, strongly convex, about one and a third times as tall as the gena; upper lobes rather widely separated by about twice the breadth of an upper lobe.

Pronotum slightly trapezoidal; about one and a half times as broad, across the lateral tubercles, as long; sides slightly widening to the lateral tubercles, which are very broad basally, acutely spinous apically, rather strongly latero-posteriorly directed, and placed at the basal quarter, thence strongly constricted to the base; disc rather convex, feebly depressed medially posterior transverse sulcus broad, shallow and continued laterally below the base of the lateral tubercles; posterior sulcus with a single row of moderate-sized, coarse, punctures; the rest fairly finely and closely alutaceously punctured, matt. Scutellum more or less semicircular; finely and fairly closely punctured.

Elytra elongate-ovate, almost parallel-sided to about the middle, thence very broadly rounded to the apices which are each separately, broadly rounded; disc moderately depressed premedially, centro-basal area slightly tumid; distinctly and rather coarsely punctured, rather sublinearly, becoming much finer and slightly sparser towards the apex and ceasing at about the apical quarter, although continued rather further subsuturally; the interstices fairly closely micropunctured.

Underside finely and variably closely punctured, except the submentum which is almost impunctate and nitid. Prosternal process linear, lacking posteriorly, the coxae contiguous. Mesosternal process linear, the coxae almost contiguous; almost plane, very feebly declivous anteriorly; acute apically. Apical ventrite short, about equal in length to the preapical, more or less semicircular; broadly truncate apically, shortly and fairly densely fringed. Pygidium short, very broad, broadly rounded and moderately fringed apically.

Legs moderately elongate; profemora strongly clavate; meso- and metafemora pedunculo- clavate, the former strongly, the latter less swollen. Tibiae normal, anterior rather short and fairly robust, the others more elongate and slender. Tarsi elongate and slender, the anterior rather broadened; first segment of metatarsi about one and a half times as long as the following two segments united.

FEMALE: Unknown.
LENGTH: 4.5 mm. BREADTH: 1.4 mm.
LOCALITY: Guatemala: Chichicastenango (6000 feet) (7. VIII. 1947, Cols. C. & P. Vaurie) (F. Johnson, donor).

Holotype (male) in the American Museum of Natural History, New York. Unique.

This new species is possibly most closely allied to *Urgleptes euprepes* Bates, from which it differs at first glance in the pubescence being grey not ochraceous-grey, the basal elytral dark fascia extended posteriorly along the suture and the preapical bilobed macula distinctly joined laterally to the postmedian fascia.

At first glance in colouration and markings it appears almost identical with *Urgleptes knulli* Dillon, except that the three subparallel premedian elytral maculae are more distinct. It differs however through the rounded elytral apices, the contiguous procoxae, the linear mesosternal process, and the varying comparative lengths of antennal segments, amongst other differences.

Urgleptes laxicollis sp. nov.

Figure 2.

MALE: Ferruginous, with greyish pubescence, leaving darker markings; elytra with the derm blackish in the main away from the basal declivity. Head

thinly greyish pubescent, rather denser round the eyes. Pronotum greyish pubescent, with a rather narrow vitta on each side medially, these broadly unite on the apical third and then extend somewhat posteriorly along the midline, each vitta extended laterally along the basal sulcus to join a rather vague oblique vitta which runs above the lateral tubercles. Scutellum light ferruginous, rather darker apically, very thinly greyish pubescent. Elytra with the basal declivity and base to about the basal twelfth subsuturally, light ferruginous; the suture narrowly light ferruginous, more broadly posteriorly, the premedian maculae light ferruginous; in general pitchy, with very sparse brownish pubescence; each elytron with narrow greyish pubescent lines about as follows:—a small macula centro- basally with a narrow line to subhumerally on the disc, curving and continuing to about the centre of the disc at about the basal third, crossing at about the middle of its length, a narrow line lying from the side of the disc at about basal quarter to centro-discally at about the basal fifth, then curving to the suture at about the basal quarter; about medially a very oblique, moderately broad, greyish fascia from the edge of the disc at about apical third, to suture at about the basal third; a similar, broad, very oblique fascia on the apical half from edge of the disc at about apical seventh to suture at about apical third, which curves posteriorly a little along the margin and has two short anterior projections; also a greyish subsutural, greyish, macula a little postmedially. Ventral surface ferruginous, fairly thinly greyish pubescent. Antennae light, rather flavous, ferruginous, the apex of the scape, the second segment completely, and the base and apex of the following segments distinctly black; thinly greyish pubescent. Legs ferruginous, the apical part of the pro- and mesofemora blackish in part; about the apical half or so of the tibiae dark ferruginous or blackish; tarsi blackish, except about the basal two-thirds of the first segment; thinly greyish pubescent.

Small, rather broadly elongate-ovate, a little subdepressed. Antennae elongate and slender, about twice as long as the body; scape sparsely preapically, and segments two to four rather densely and closely, setose beneath; scape a little, regularly, swollen, fairly elongate, extending to the middle of the basal sulcus of the pronotum; third segment about one and a quarter times as long as the scape; fourth segment slightly shorter than the third, slightly longer than the scape; fifth segment distinctly shorter than the fourth, following segments very gradually decreasing to the apical (eleventh) segment; the segments fairly finely and closely punctured. Antennal tubercles slightly swollen, slightly raised, very broadly and moderately shallowly foveate medially behind the tubercles. Frons fairly large, about quadrate, very feebly narrowed above between the eyes; rather feebly convex, with a fine, rather indistinct, median longitudinal line; head very finely and closely microgranular and micropunctured. Eyes not very large; lower lobe of only moderate size, rather rotundate, about as broad as long, about one and a quarter times as tall as the gena; upper lobes moderately approaching, separated by about one and a half times the breadth of an upper lobe.

Pronotum strongly transverse, about one and three-quarter times as broad

as long; the sides rather strongly, rather planately tumescent medially, to project laterally about as far as the level of the apex of the lateral spine, to which the sides are a little constricted; lateral spines placed only very slightly behind the basal third, small, rather slender and acute, thereafter strongly constricted to the base; disc slightly convex; posterior transverse sulcus very broad and shallow, extended sublaterally past the base of the tubercles, with a single row of distinct, fairly large, coarse, fairly close punctures, which cease a little before reaching the base of the lateral tubercles; the rest completely closely microgranular and micropunctured, rather matt. Scutellum very broadly subtriangular rather short; very finely and only fairly closely punctured.

Elytra rather broadly elongate-ovate; slightly widening laterally to about the middle, thence very broadly rounded to the apices, which are each separately, rather narrowly rounded; disc very feebly depressed premedially, slightly swollen centro-basally; distinctly, coarsely and fairly closely, rather irregularly punctured, becoming distinctly smaller and more sparse from a little before the middle, and almost ceasing, except for an occasional large puncture, at about the apical third; the interstices and rest of the elytra extremely finely and closely punctured.

Underside very finely and mainly closely punctured, except the submentum which is impunctate and nitid. Prosternal process extremely narrow, sublinear, the coxae almost contiguous; almost plane, much lower than the coxae. Mesosternal process very narrow, but about three times as broad as the prosternal process, about a seventh or eighth as broad as a mesocoxal cavity; feebly explanate apically, the apex narrowly truncate; rather distinctly longitudinally canaliculate; very gradually declivous anteriorly. Apical ventrite fairly short about one and a half times as long as the preapical, more or less semicircular, the apex moderately broadly truncate or feebly emarginate, moderately fringed; (the holotype male with a few vannate, irregular, preapical, longitudinal corrugations, not present in paratype males). Pygidium short, broad, very broadly rounded, almost truncate apically.

Legs moderately elongate; profemora strongly clavate, meso- and metafemora strongly pedunculate, the posterior less swollen. Tibiae normal, the anterior with a small, obtuse, premedian, ventral tooth; the intermediate and posterior more slender. Tarsi elongate and slender, the anterior somewhat broadened; first segment of metatarsi about one and a third times as long as the following two segments united.

FEMALE: Unknown.

LENGTH: 3.1 - 4.4 mm. BREADTH: 1.2 - 1.7 mm.

LOCALITY: Guatemala: Sac (atepéquez?), Reunion (4000 feet.) (22. VIII. 1947, Cols. C. & P. Vaurie). (F. Johnson, donor). (Holotype, ♂). Such (iate?) Moca (3000 feet). (31. VIII. 1947, Cols. C. & P. Vaurie). (F. Johnson, donor). (Paratype ♂). Such (iate?) Variedades (500 feet). (1. IX. 1947, Cols. C. & P. Vaurie). (F. Johnson, donor) (Paratype ♂).

Holotype (male) and two Paratypes (males) in the American Museum of Natural History, New York. (1 ♂ Paratype placed in the author's collection).

Through the strongly transverse pronotum, this new species is allied to *Urgleptes tumidicollis* Bates, from which it may be distinguished by the elytral base being distinctly lighter in colour than the rest, the pronotum distinctly bivittate medially and other differences.

Urgleptes elaineae sp. nov.

Figure 3.

FEMALE: Dark ferruginous with lighter ferruginous in places; with grey pubescence in parts, on the elytra outlining maculae. Head pitchy-black, with a little, extremely sparse greyish pubescence. Pronotum pitchy-ferruginous, with a single, median, longitudinal, complete vitta, which is fairly narrow, and narrows to the apex; thinly greyish sublaterally. Scutellum ferruginous, very thinly greyish pubescent. Elytra dark ferruginous or pitchy-ferruginous, except a premedian oblique row of subparallel, elongate-oval maculae which are lighter ferruginous; a small macula of grey pubescence centro-basally, and a short narrow vitta of grey behind the scutellum, subsuturally; on the basal half on each elytron an oblique row of oval ferruginous maculae from post-humerally to the suture about the middle, distinctly and fairly broadly connectively annulate with grey, which continues on the epipleurum to the margin; a slightly postmedian, blackish, oblique, irregular fascia and another at the apical quarter, both separated and bordered with grey pubescence, and the extreme apex greyish, narrowly uniting marginally and suturally with the previous grey fascia. Ventral surface blackish, with thin grey pubescence. Antennae pitchy-ferruginous, the scape lighter basally; very thinly greyish-brown pubescent. Legs dark ferruginous; coxae, base of femora, and base of tibiae (less distinctly), light ferruginous; very thinly greyish pubescent.

Small, elongate-ovate, a little subdepressed. Antennae elongate and slender (missing after the sixth segment); scape preapically and segments to the fourth very sparsely setose below; scape moderately strongly and regularly swollen to the apex, elongate, not quite extending to the pronotal base; third segment equal in length to the scape; fourth segment about a tenth longer than the third; fifth and sixth very gradually decreasing in length; (remaining segments missing); segments finely and closely punctured. Antennal tubercles slightly swollen, only a little raised, broadly and very shallowly concave between; slightly, broadly foveate medially behind the tubercles. Frons large, about quadrate, a little narrowed above between the eyes, moderately strongly convex, with a fine median longitudinal line; head completely very finely and fairly closely granular, micropunctured, rather matt. Eyes moderately large; lower lobe fairly large, somewhat subtriangular, about one and a half times as long as broad, about twice as tall as the gena; upper lobes moderately closely approaching above, separated by about one and a quarter times the breadth of an upper lobe.

Pronotum rather strongly trapezoidal, about one and two-third times as broad, across lateral tubercles, as long; sides fairly strongly widening to the lateral tubercles which are placed at about the basal quarter, are very broad based and end in a fairly long acute spine, strongly constricted thereafter to the base; disc slightly convex; posterior transverse sulcus broad, very shallow and continued sublaterally past the base of the lateral tubercles, with a single row of fairly small, rather close, coarse punctures; the rest completely very finely and closely granular, micropunctured, rather matt. Scutellum almost quadrate, but very broadly rounded apically; fairly finely and moderately closely punctured.

Elytra elongate-ovate, slightly widening laterally to about the middle, thence very broadly rounded to the apices which are very slightly obliquely truncate, both sutural and marginal angles rounded; disc feebly depressed premedially, scarcely swollen centrobasally; distinctly fairly coarsely and fairly closely, rather irregularly punctured, becoming distinctly smaller and somewhat more sparse towards the apex, and ceasing at about the apical seventh, the interstices and rest fairly closely micropunctured.

Underside finely and closely punctured, except the submentum which is impunctate and nitid. Prosternal process extremely narrow, almost linear, coxae almost contiguous, about plane, much lower than the coxae. Mesosternal process very narrow, very slightly broader than the prosternal process, strongly narrowing to the apex which is acute; slightly declivous anteriorly. Apical ventrite fairly short, about one and a half times as long as the preapical, rather broadly subconical; fairly broadly, slightly emarginate apically. Pygidium rather short, broad, moderately truncate apically.

Legs only moderately elongate; profemora strongly clavate, meso- and metafemora pedunculo-clavate, the latter much less strongly swollen. Tibiae normal, the posterior most slender. Tarsi moderately elongate and slender, the anterior a little broader; first segment of metatarsi about twice as long as the following two segments united.

MALE: Unknown.

LENGTH: 4.4 mm. BREADTH: 1.5 mm.

LOCALITY: Guatemala: B. V. P., San Jerónimo (3000 feet) (27. VII. 1947, Cols. C. & P. Vaurie), (F. Johnson, donor).

Holotype (female) in the American Museum of Natural History, New York. Unique.

This new species is probably most closely allied to *Urgleptes clerula* Bates to which it is very similar in markings, although the premedian row of three subparallel dark maculae is much more disinct. It may be immediately distinguished, however, by the elytral apices which have neither angle armed, whereas in *clerula* Bates, the apices are emarginate, with the marginal angle rather strongly and acutely produced.

Urgleptes dorotheae sp. nov.

Figure 4.

MALE: Ferruginous, with fairly dense greyish pubescence, and dark brown markings. Head blackish ferruginous, very thinly greyish pubescent. Pronotal disc with four dark brown vittae, two very broad median, complete, and a narrower, rather curved one on each side, above the lateral tubercles; prothorax broadly dark brown below. Scutellum thinly greyish pubescent, the derm darker ferruginous apically. Elytra rather densely greyish pubescent, each elytron with dark brown, thinly pubescent maculae as follows, none of which reach the suture, which is narrowly grey pubescent:—a centro-basal, subscutellar, macula, which is narrowly prolonged posteriorly to about the basal quarter; a large elongate lateral macula from the humerus anteriorly to about the basal two-fifths, touching the margin and extending a little onto the disc; a large, very oblique, irregular, transverse fascia from premedially suturally to margin at about the apical third or two-sevenths, which has two anterior discal projections and two posterior, curved posteriorly, marginally, almost to the apex and thence curving back forwards on the disc to a little past the apical fifth. Ventral surface dark ferruginous, thinly greyish pubescent. Antennae dark ferruginous, the basal segments a little lighter; thinly greyish or greyish-brown pubescent. Legs dark ferruginous, coxae and base of femora light ferruginous proximal half of tibiae ferruginous; thinly greyish pubescent.

Small, elongate, ovate, only very feebly subdepressed. Antennae elongate and slender, about twice as long as the body; scape preapically and segments to the fourth sparsely, but distinctly, setose below; scape moderately and regularly swollen to the apex, moderately elongate, extending to about the basal fifth, or basal sulcus, of the pronotum; third segment about one and a third times as long as the scape; fourth segment only very slightly shorter than the third; fifth segment distinctly shorter than third, about equal to the scape; following segments subequal to fifth, extremely gradually decreasing to the apex, the apical (eleventh) segment, rather more distinctly shorter than the preapical; segments finely and fairly closely punctured. Antennal tubercles moderately swollen, moderately raised, broadly and moderately concave between; feebly and broadly foveate medially behind the tubercles. Frons large, about quadrate, very feebly narrowed above between the eyes, moderately convex, with a very fine median longitudinal line; head very finely and closely punctured, somewhat alutaceously on the vertex. Eyes small; lower lobe small, rather rotundate, fairly strongly convex, about equal in height to the gena; upper lobes widely separated by about two and a half times the breadth of an upper lobe.

Pronotum trapezoidal, strongly transverse, twice as broad, across the lateral tubercles which are placed at about the basal third, and very broad based, short, and with a small acute apex, strongly constricted behind to the base; disc slightly convex; posterior transverse sulcus broad, very shallow and continued sublaterally past the base of the lateral tubercles, with a single row

of close, rather coarse punctures, the rest completely finely and closely granular, micropunctured and rather matt. Scutellum subtriangular, fairly narrowly rounded apically; finely, but only moderately closely punctured.

Elytra elongate-ovate, slightly widening laterally to about the middle, thence very broadly rounded to the apices, which are separately fairly broadly rounded; disc moderately convex, extremely feebly depressed premedially, very feebly swollen centro-basally; distinctly, coarsely, and rather closely, mainly rather irregularly punctured, becoming rather smaller and a little more sparse towards the apex and ceasing at about the apical ninth; the interstices and rest fairly closely micropunctured.

Underside very finely and closely punctured, except the submentum which is impunctate and nitid. Prosternal process extremely narrow, sublinear, about a twelfth or less, the breadth of a procoxal cavity, the coxae almost contiguous, a little rounded, but much lower than the coxae. Mesosternal process extremely narrow, linear, very slightly narrower than the prosternal process, coxae almost contiguous, almost plane, but slightly depressed medially, apex narrowly acute. Apical ventrite fairly short, about one and a third times as long as the preapical, more or less semicircular; moderately broadly truncate apically, shortly fringed. Pygidium fairly short, broad, broadly rounded apically.

Legs of moderate length; profemora strongly clavate, meso- and metafemora pedunculo-clavate, the former strongly, the latter less swollen. Tibiae normal; moderately elongate and fairly slender. Tarsi fairly elongate and slender, the anterior slightly broadened; first segment of metatarsi about one and a half times as long as the following two segments united.

FEMALE: Similarly coloured to the male. Pronotum a little less strongly transverse, about one and three-quarters as broad as long. Apical ventrite feebly subconical, more narrowly truncate apically. (Antennae unfortunately broken after the sixth segment).

LENGTH: 3.2 - 3.8 mm. BREADTH: 1.1 - 1.3 mm.
LOCALITY: Guatemala: Nebaj (6000 feet) (10. VIII. 1947, Cols. C. & P. Vaurie) (F. Johnson, donor). (2♂, 1♀).

Holotype (male), Allotype (female) and one Paratype (male) in the American Museum of Natural History, New York. (The ♂ paratype placed in the author's collection).

This new species is quite conspicuous in the almost completely finely granular pronotal disc, a character found in a few other species of *Urgleptes* Dillon. Both pro- and mesocoxae are almost contiguous. It is propably most closely allied to *Urgleptes ornatissima* Bates, to which it is very similar in general markings, but from which, apart from some distinct macula variations, it differs in having the elytral apices rounded, not truncate, and the pronotum more transverse.

Urgleptes vauriearum sp. nov.

Figure 5.

MALE: Ferruginous, with greyish pubescence, with dark brown, thinly pubescent markings. Head dark ferruginous, thinly greyish pubescent, denser round the eyes and medially on the vertex. Pronotal disc with four, broad, dark ferruginous vittae, two median, almost complete, but not extending past the basal sulcus, and a lateral on each side, curved above the lateral tubercles. Scutellum dark brown. Elytra with an oblique, blackish-ferruginous, post-median fascia from between the apical four-ninths and third suturally, widening laterally to between the apical two-ninths and sixth, with a short anterior projection at the edge of the disc; the rest of the elytra variegated greyish pubescent, before and after the fascia, on the discs, agglomerating, on a little more than the anterior half, to form two, rather broken, irregular vittae, and rather densely along the anterior border of the fascia. Ventral surface black, thinly grey pubescent. Antennae ferruginous, the apices of the segments from the third blackish, the apical segments more completely darkened; very thinly greyish pubescent. Legs pitchy, base of femora ferruginous, and basal half of tibiae dark ferruginous; thinly greyish pubescent.

Fairly small; elongate-ovate, subdepressed. Antennae elongate and slender, almost two and a half times as long as the body; scape preacically and segments to the fourth very sparsely setose beneath: scape moderately and regularly swollen to the apex, moderately elongate, extending almost to the pronotal base; third segment very slightly longer than the scape; fourth segment about one and a sixth times as long as the third; fifth segment about equal to the third; sixth and seventh very gradually decreasing; eighth to eleventh (apical) very gradually increasing in length; segments finely and fairly closely punctured, the scape rather distinctly more coarsely. Antennal tubercles only feebly swollen, scarcely raised, rather planate above; broadly and extremely shallowly concave between; broadly and feebly foveate medially behind the tubercles. Frons large, transverse, about one and a third times as broad as long, feebly narrowed above between the eyes; moderately convex, with a fine median longitudinal line, which is more distinct superiorly; head finely and closely, alutaceously punctured, somewhat matt. Eyes small; lower lobe rather small, somewhat rotundately subtriangular, fairly strongly convex, about one and a quarter times longer than broad, about one and a quarter times as tall as the gena; upper lobes widely separated by about three times the breadth of an upper lobe.

Pronotum somewhat trapezoidal, only slightly more than one and a third times as broad, across the lateral tubercles, as long; the sides slightly rotundate and widening to the lateral tubercles which are placed at about the basal third, are swollen and extremely broad based, obtuse with a very small acute apical spine, strongly constricted behind to the base; disc a little convex, slightly subdepressed medially; posterior transverse sulcus broad, very shallow,

continued sublaterally past the base of the tubercles, with a single row, doubled medially, of fairly close, rather coarse punctures, the rest completely finely and closely granular, distinctly more sparse on a narrow median longitudinal band, micropunctured and rather matt. Scutellum subtriangular, extremely broadly rounded, almost truncate, apically, a little swollen laterally and apically; very finely and fairly closely granular and micropunctured.

Elytra elongate-ovate, subdepressed, somewhat narrowly rounded laterally at the junction of disc and epipleura, more or less parallel-sided for about the basal half, thence very broadly rounded laterally to the apices, which are each separately rather narrowly rounded; disc slightly convex, slightly depressed premedially, a little swollen centro-basally; distinctly, coarsely and fairly closely, rather irregularly punctured, becoming slightly smaller and slightly sparser towards the apex, which they almost reach, the interstices and rest micropunctured.

Underside finely and closely punctured, except the submentum which is impunctate and nitid. Prosternal process linear, the coxae almost contiguous, more or less plane, much lower than the coxae. Mesosternal process extremely narrow, linear medially, acute apically, coxae almost contiguous, about plane above, feebly declivous anteriorly. Apical ventrite more or less semicircular, slightly longer than the preapical, moderately broadly truncate apically, shortly fringed. Pygidium short, broad, moderately truncate apically.

Legs fairly elongate; profemora strongly clavate, meso- and metafemora pedunculate, the latter a little less strongly swollen than the intermediate. Tibiae normal, the anterior most robust and rather distinctly bisinuate below, the intermediate and posterior rather elongate and slender. Tarsi fairly elongate and slender, the anterior a little broadeded; first segment of metatarsi about one and two-thirds as long as the following two segments united.

FEMALE: Unknown.
LENGTHS 5.3 mm. BREADTH: 1.7 mm.
LOCALITY: Guatemala: Nebaj (6000 feet) (10. VIII. 1947, Cols. C. & P. Vaurie) (F. Johnson, donor).

Holotype (male) in the American Museum of Natural History, New York. Unique.

I have named this species after C. & P. Vaurie who have collected so much interesting material in Central America.

This new species is perhaps rather divergent in the genus *Urgleptes*, through the pronotal shape and perhaps also through the comparatively more strongly swollen metafemora. It is possibly fairly closely allied to *Urgleptes charilla* Bates, from which it conspicuously differs through the vague, marmorated, grey elytral pubescence and the shape of the postmedian dark fascia, as well as a number of structural details.

Urgleptes sinuosa sp. nov.

Figure 6.

FEMALE: Derm pitchy in the main, elytra ferruginous in part mainly where grey pubescent. Head pitchy-black with thin grey pubescence. Pronotum pitchy-black with three, rather vague, thinly pubescent, grey vittae, one median strongly broadened medially and enclosing here an oval pitchy macula, and a lateral one on each side above the lateral tubercles; some thinner grey pubescence on the tubercles and sublaterally. Scutellum ferruginous, thinly grey pubescent. Elytra mainly pitchy with thin grey pubescence; with lighter ferruginous markings, with dense grey pubescence as follows:-on each elytron a broad sinuous band form over the humerus to the middle of the disc at about the basal third, thence curving outwards to the border of the disc at between the apical third and apical quarter; also on the apical half discally a number of conglomerate maculae which form a larger irregular one at between the apical third and seventh; vaguely, broadly, lighter on the lateral declivity and the suture narrowly lighter. Ventral surface dark ferruginous, fairly thinly grey pubescent. Antennae light ferruginous, the scape wholly and apices of the remaining segments blackish; very thinly greyish pubescence. Legs dark ferruginous, base of femora and basal half of tibiae lighter ferruginous; thinly grey pubescent.

Small, elongate-ovate, a little subdepressed. Antennae elongate and slender (missing after the eighth segment), exceeding the elytral apex after the fifth segment; scape preapically and segments to the fourth sparsely setose below; scape moderately and regularly swollen to the apex, elongate, extending almost to the pronotal base; third segment about a sixth longer than the scape; fourth segment about a twelfth longer than the third; fifth segment about equal to the scape, following segments, to the eighth at least, gradually decreasing (segments missing after the eighth); segments fairly finely and closely punctured. Antennal tubercles moderately swollen and moderately raised, broadly, obtusely and moderately V-shaped between; scarcely foveate behind the tubercles, although transversely, distinctly marked at the junction of the antennal tubercles and vertex. Frons large, about quadrate, only feebly narrowed above between the eyes; moderately strongly convex, with an extremely fine median longitudinal line, which is almost obsolete medially; frons moderately finely and only fairly closely punctured; vertex closely microgranular and somewhat matt. Eyes rather small; lower lobe small, a little subquadrate, about as long as broad, scarcely as tall as the gena; upper lobes rather small and narrow, widely separated by about three times the breadth of an upper lobe.

Pronotum feebly trapezoidal, about one and two-thirds as broad, across the lateral tubercles, as long; sides slightly widening to the lateral tubercles which are placed at about the basal quarter, are moderately broad basally and produced in an acute spine which is rather strongly latero-posteriorly directed, strongly constricted thereafter to the base; disc slightly convex; posterior trans-

verse sulcus very broad and shallow, continued sublaterally past the base of the tubercles, with a single row of fairly large, moderately close punctures; the resa completely fairly closely microgranular, and fairly closely micropunctured between, a little matt. Scutellum subtriangular, about rectangularly rounded apically; fairly finely and moderately closely punctured.

Elytra elongate-ovate, widening a little laterally to about the middle, thence broadly rounded to the apices which are obliquely truncate, the sutural angle broadly rounded, the marginal about rectangularly rounded; disc feebly depressed premedially, only very feebly swollen centro-basally; rather coarsely, rather irregularly and only moderately closely punctured, the punctures becoming smaller towards the apex, the interstices moderately closely micropunctured.

Underside very finely and fairly closely punctured, except the submentum which is impunctate and nitid. Prosternal process sublinear, coxae almost contiguous, almost plane, much lower than the coxae. Mesosternal process extremely narrow, distinctly constricted medially where it is scarcely wider than the prosternal process, gradually and slightly explanate to its apex which is narrowly truncate; almost plane above, slightly declivous anteriorly. Apical ventrite rather broadly subconical, about one and a half times as long as the preapical; moderately broadly, feebly emarginate apically. Pygidium of moderate length, broad, moderately broadly almost truncate apically.

Legs only moderately elongate; profemora strongly clavate, meso-and metafemora pedunculo-clavate, the latter much less swollen. Tibiae normal, not very elongate, fairly slender. Tarsi moderately elongate, the anterior somewhat broadened, the posterior slender; first segment of metatarsi about one and three-quarter times as long as the following two segments united.

MALE: Unknown.
LENGTH: 4.8 mm. BREADTH: 1.6 mm.
LOCALITY: Guatemala: Nebaj (6000 feet) (10. VIII. 1947, Cols. C. & P. Vaurie) (F. Johnson, donor).

Holotype (female) in the American Museum of Natural History, New York. Unique.

This new species is possibly most closely allied to *Urgleptes ornatissima* Bates, from which it differs conspicuously however in the much less distinct elytral markings, which are not densely pubescent, although arranged in a very similar fashion, the pubescence being very sparsely grey, not densely ochraceous.

In general appearance somewhat like *Lepturgotrichona stigmatica* Bates, from which it differs through the elytra not having erect setae and in the basal maculation.

Possibly a rather aberrant species in the genus through the comparatively small eyes and widerly separated upper lobes.

Urgleptes bimaculata sp. nov.

Figure 7.

MALE: Dark ferruginous; head, anterior and posterior borders of the pronotum, base of elytra very narrowly and part of the suture, generally rather lighter ferruginous with greyish and brownish pubescence, the former mainly on the elytra. Head fairly dark ferruginous, with thin brownish pubescence, and some greyish pubescence round the eyes. Pronotum dark ferruginous, the base and apex generally lighter; rather thinly greyish pubescent; disc with five dark vittae, a very narrow median one on about the basal two-thirds; one on each side of the middle of the disc, extending to about the basal sulcus, strongly broadening anteriorly and sometimes narrowly connected, and laterally above each lateral tubercle an oblique, rather curved, narrower, shorter vitta. Scutellum in general lighter ferruginous, with the sides and apex dark ferruginous; very thinly greyish-brown pubescent. Elytra with the entire disc from about basal sixth, continued narrowly to latero-anteriorly round the centro-basal tumid area, to about the apical five-elevenths, fairly densely greyish pubescent, somewhat broken laterally; the apical five-elevenths rather variegated greyish pubescent, leaving a few dark maculae more distinct, two subparallel from the basal five-elevenths, and a preapical conjoint group of three or four; about the apical three-quarter of the margin greyish marmorated, becoming rather denser posteriorly; each elytron with a fairly small, rather distinct, densely white pubescent macula at the edge of disc, just above the epipleurum at about the apical two-fifths. Ventral surface ferruginous, with thin, somewhat flavous-grey pubescence, which is rather denser on the sides of the sterna. Antennae with segments from the second ferruginous, with their apices, and almost the whole of the scape blackish; thinly greyish pubescent. Legs dark ferruginous or pitchy; base of the femora rather light ferruginous; about the basal half of the tibiae a little lighter; thinly somewhat flavous-grey pubescent.

Small to fairly small, elongate-ovate, subdepressed. Antennae elongate and slender, slightly more than two and a half times as long as the body; scape preapically and segments to the fourth sparsely setose below; scape moderately swollen, extending to the basal sulcus of the pronotum; third segment almost one and a quarter times as long as the scape fourth segment very slightly larger than the third; fifth to seventh segments extremely gradually decreasing; eighth and ninth segments extremely gradually increasing; tenth segment slightly shorter than ninth; eleventh, apical, segment slightly longed than the preapical; segments fairly finely and fairly closely punctured. Antennal tubercles a little swollen, slightly raised, broadly and very obtusely V-shaped between; broadly and rather shallowly foveate medially behind the tubercles. Frons fairly large, very slightly transverse, about one and a tenth broader than long; slightly narrowed above between the eyes, moderately convex, with a fine median longitudinal line; head with frons extremely finely microgranular, vertex more coarsely micro-granular and micropunctured; somewhat matt. Eyes moderately large; lower lobe

of moderate size, almost quadrate, moderately strongly convex, almost twice as tall as the gena; upper lobes comparatively fairly large, moderately separated by about one and a half times the breadth of an upper lobe.

Pronotum trapezoidal, about one and three-quarters times as broad, across the lateral tubercles, as long; sides widening a little to the lateral tubercles, which are placed at about the basal third, are very broad based and slightly tumescent anteriorly, of moderate size, with a rather small fairly acute apex, thereafter strongly constricted to the base; disc slightly convex, slightly subdepressed medially; posterior transverse sulcus very broad, shallow and continued sublaterally past the base of the tubercles, with a single row of only moderately coarse, only fairly close punctures, the rest almost completely, closely, microgranular and micropunctured, except a very narrow longitudinal band on about the posterior half of the disc; a little matt. Scutellum fairly large, somewhat subtriangular, sometimes of rather elongate appearance, broadly rounded apically; fairly closely microgranular and micropunctured; rather matt.

Elytra elongate-ovate; very slightly narrowing to about the middle, thence broadly rounded to the apices which are rather strongly obliquely truncate, the sutural angle broadly rounded, the marginal not produced, about rectangular; disc rather distinctly subdepressed, rather extensively slightly depressed premedially, the centro-basal area broadly, moderately tumescent; distinctly, rather coarsely, only fairly closely punctured, mainly rather irregularly, a little sublinearly infrahumerally, the punctures on the premedian depressed area distinctly less coarse than those basally and laterally, becoming slightly smaller towards the apex, and ceasing at about the apical fifth; interstices and rest very finely and fairly closely punctured.

Underside very finely and closely punctured, except the submentum which is almost impunctate and nitid. Prosternal process linear medially, coxae almost contiguous; almost plane, much lower than the coxae. Mesosternal process extremely narrow, linear or sublinear, coxae almost or not quite contiguous; almost plane, much lower than the coxae. Mesosternal process extremely narrow, linear or sublinear, coxae almost or not quite contiguous; very slightly concave above, anterior border shortly and strongly declivous, almost vertical; very gradually and extremely slightly explanate to the apex, which is very narrowly truncate. Apical ventrite fairly short, slightly longer than the preapical, more or less semicircular, the apex very broadly almost truncate, but very feebly rounded; moderately fringed. Pygidium fairly short, broad, moderately rounded apically; sparsely microgranular; shortly fringed.

Legs elongate; profemora strongly clavate, meso- and metafemora pedunculate, the latter a little less strongly swollen. Tibiae normal, the intermediate and posterior elongate and slender. Tarsi elongate, the anterior a little broadened, the others slender, the posterior most so; first segment of the metatarsi about one and two-third times as long as the following two segments united.

FEMALE: Similarly coloured to the male, except the proximal half of tibiae more distinctly light ferruginous and the basal two-thirds or so of the first metatarsal segment light ferruginous. Slightly broader in build than the

male. Antennae about two and a quarter times as long as the body; third segment slightly shorter than the third; fifth segment slightly shorter than fourth, the following segments to the tenth each about equal to the fifth, the apical segment slightly longer than the preapical.

Pronotum very slightly more transverse than in the male.

Prosternal process very narrow, not quite sublinear, about one tenth as broad as a procoxal cavity; rather strongly canaliculate; more or less plane, much lower than the coxae. Mesosternal process very narrow, about twice as broad as the prosternal process, but apparently a little variable, about one-seventh to one-ninth as broad as a mesocoxal cavity; very slightly (Allotype) or not (Paratype) explanate apically, the apex fairly narrowly to narrowly truncate; somewhat canaliculate; feebly concave above, slightly and shortly declivous anteriorly. Apical ventrite subconical, about one and two-third times as long as the preapical; moderately broadly truncate apically, with a few, very sparse, elongate, preapical black setae. Pygidium rounded apically; very sparsely, very finely microgranular.

Legs slightly shorter than in male. First segment of metatarsi not quite one and two-thirds as long as the following two segments united.

LENGTH: 4.5 - 6.4 mm. BREADTH: 1.5 - 2.2 mm.

LOCALITY: Guatemala: Nebaj (6000 feet) (10. VIII. 1947, Cols. C & P. Vaurie) (F. Johnson, donor) (Holotype, ♂, Allotype, ♀, 2 Paratypes, ♂, 1 paratype, ♀). *Idem data* (9. VIII. 1947) (2 Paratypes, ♂) . Chichicastenango (6000 feet) (7. VIII. 1947, Cols. C. & P. Vaurie) (F. Johnson, donor). (Paratype, ♂). Cunen (6000 feet) (11. VIII. 1947, Cols. C. & P. Vaurie) (F. Johnson, donor). (Paratype, ♀).

Holotype (male), Allotype (female) and seven Paratypes (5 males, 2 females) in the American Museum of Natural History, New York. (2♂ and 1 ♀ Paratypes placed in the author's collection).

This distintive new species may be separated from *Urgleptes clerula* Bates, which is slightly similar in appearance, by the elytral marginal apical angle not produced and lacking a distinct postmedian dark fascia, amongst other differences.

Urgleptes bicolorata sp. nov.

Figure 8.

FEMALE: In general dark ferruginous or pitchy, the elytra with the discal anterior half, extended to the margin between slightly premedially and the apical two-fifths, fairly light ferruginous, distinctly contrasting with the rest. Otherwise more or less immaculate; the head dark ferruginous, thinly grey pubescent round the eyes; pronotum very thinly greyish laterally, a little condensed into a narrow vague vittae laterally on the disc; scutellum ferruginous,

slightly darker apically; elytra very thinly and mostly indistinctly grey pubescent, slightly denser humerally, premedially and latero-medially on the disc. Ventral surface ferruginous, the pro- and mesosterna blackish laterally, and the posterior borders of the abdominal ventrites blackish, the apical almost completely; thinly grey pubescent. Antennae ferruginous basally, the inner side of the scape and the segments gradually becoming blackish or pitchy towards the apex. Legs pitchy, the base of the femora, and vaguely the basal half of the tibiae, ferruginous; very thinly greyish pubescent.

Small, elongate-ovate, rather subdepressed. Antennae elongate and slender, a little more than twice as long as the body (segments missing after the ninth); scape preapically and segments to fourth very sparsely setose below; scape moderately swollen, moderately elongate, extending to the basal sulcus of the pronotum; third segment about one and a ninth longer than the scape; fourth segment about one and a seventh longer than the third; fifth to seventh segments very gradually decreasing; eighth and ninth slightly more elongate, about equal to each other; (following segments missing); segments finely and fairly closely punctured. Antennal tubercles only rather feebly swollen, only slightly raised, broadly and very shallowly concave between; broadly and moderately foveate medially behind the tubercles. Frons large, transverse, about one and a quarter times as broad as long, somewhat narrowed above between the eyes, moderately convex, with a fine, distinct, median longitudinal line; head completely, closely, microgranular and micropunctured. Eyes small; lower lobe small, subtriangular, about one and a third times as long as broad, about one and a third times as tall as the gena; upper lobes widely separated by about three times the breadth of an upper lobe.

Pronotum trapezoidal, about one and a half times as broad, across the lateral tubercles, as long; sides widening to the lateral tubercles which are placed at about the basal quarter, are very obtuse and very broad based, ending in a very small acute spine, strongly constricted thereafter to the base; disc slightly convex, feebly subdepressed medially; posterior transverse sulcus broad and shallow, and continued sublaterally past the base of the tubercles, with a single, somewhat medially irregular, row of distinct, fairly coarse, moderately close punctures; the rest completely, closely, microgranular and micropunctured, rather matt. Scutellum subtriangular, rounded apically; rather sparsely microgranular and micropunctured.

Elytra elongate-ovate, slightly widening laterally to about the middle, thence broadly rounded to the apices, which are each separately, fairly narrowly rounded; disc moderately convex posteriorly, rather subdepressed above and premedially, centro-basal area a little tumescent, the junction of disc and epipleura rather narrowly rounded; distinctly, very coarsely, not very closely, rather irregularly punctured, although a little sublinear in part, becoming smaller towards the apex and rather more sparse towards the extreme apex, the interstices fairly closely micropunctured.

Underside finely and closely punctured, except the submentum which is impunctate and nitid. Prosternal process extremely narrow, linear, the coxae

almost contiguous, plane, much lower than the coxae. Mesosternal process very narrow, extremely narrowly, linear and very acute apically, the coxae almost contiguous posteriorly, plane above, rather declivous, although shortly, anteriorly. Apical ventrite a little subconical, about one and a half times as long as the preapical, rather narrowly truncate apically, sparsely fringed. Pygidium a little elongate, extending slightly past the elytral apex, rather broad, fairly narrowly rounded apically, sparsely fringed.

Legs of only moderate length; profemora fairly strongly clavate, meso- and metafemora pedunculate, the former quite strongly swollen, the latter fairly slender. Tibiae normal, the intermediate and posterior slender. Tarsi elongate and slender, the anterior very slightly broadened; first segment of metatarsi about one and a third times as long as the following two segments united.

MALE: Unknown.

LENGTH: 4.1 mm. BREADTH: 1.2 mm.

LOCALITY: Guatemala: Nebaj (6000 feet) (10. VIII. 1947, Cols. C. & P. Vaurie) (F. Johnson, donor).

Holotype (female) in the American Museum of Natural History, New York. Unique.

This most distinctly coloured species is possibly most closely allied to *Urgleptes xantho* Bates, but lacks the comparatively dense flavous pubescence, and is quite different in the bicoloured elytra.

SUMMARY

The author describes eight new species of the genus *Urgleptes* Dillon from Guatemala. These are *pareuprepes, U. dorotheae, U. laxicollis, U. elaineae, U. sinuosa, U. bicolorata, U. bimaculata, and U. vauriearum.*

It is considered that the greatest variety of species in this genus occurs in Central America, and about fifty species are at present known.

The author states that much useful fieldwork could be done in collecting material for systematic examination, many parts of Central America being unexplored entomologically.

RESUMEN

Se describen ocho especies nuevas del género *Urgleptes* Dillon (Coleoptera, Cerambycidae) de Guatemala: *U. pareuprepes, U. dorotheae, U. laxicollis, U. elaineae, U. sinuosa, U. bicolorata, U. bimaculata* y *U. vauriearum.*

Hasta el momento el género cuenta con unas cincuenta especies, la mayor parte de las cuales se encuentran en la América Central, donde se hace necesario continuar la exploración entomológica.

REFERENCES

1. DILLON, L. S.
1956. The Nearctic components of the tribe Acanthocinini (Coleoptera, Cerambycidae). Parts 1-3. *Ann Ent. Soc. Amer.*, 49(2): 134-167, pl. 1; 49(3): 207-235, pl. 1; 49(4): 332-335, pl. 1.

2. GILMOUR, E. F.
1959. On the Neotropical Acanthocinini, V. (Coleoptera, Cerambycidae, Lamiinae); new species of *Lepturges* Bates and *Urgleptes* Dillon. *Opusc. Zool., München,* 28:1-10, figs. 1-4.

Fig. 1: *Urgleptes paucuprepes* sp. nov. ♂. Holotype.

Fig. 2: *U. laxicollis* sp. nov. ♂. Holotype.

Fig. 3: *U. elaineae* sp. nov. ♀. Holotype.

Fig. 4: *U. dorotheae* sp. nov. ♂. Holotype.

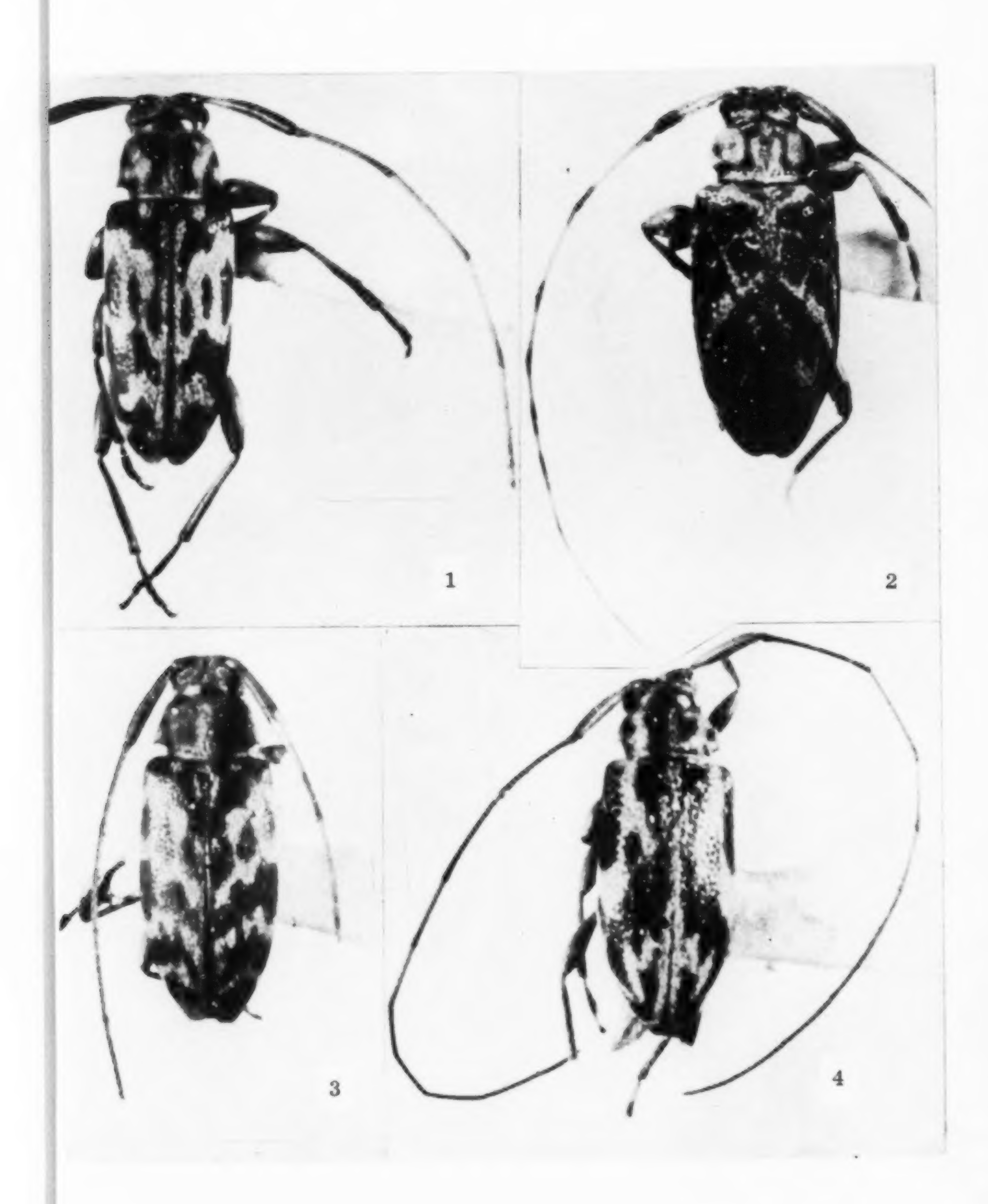
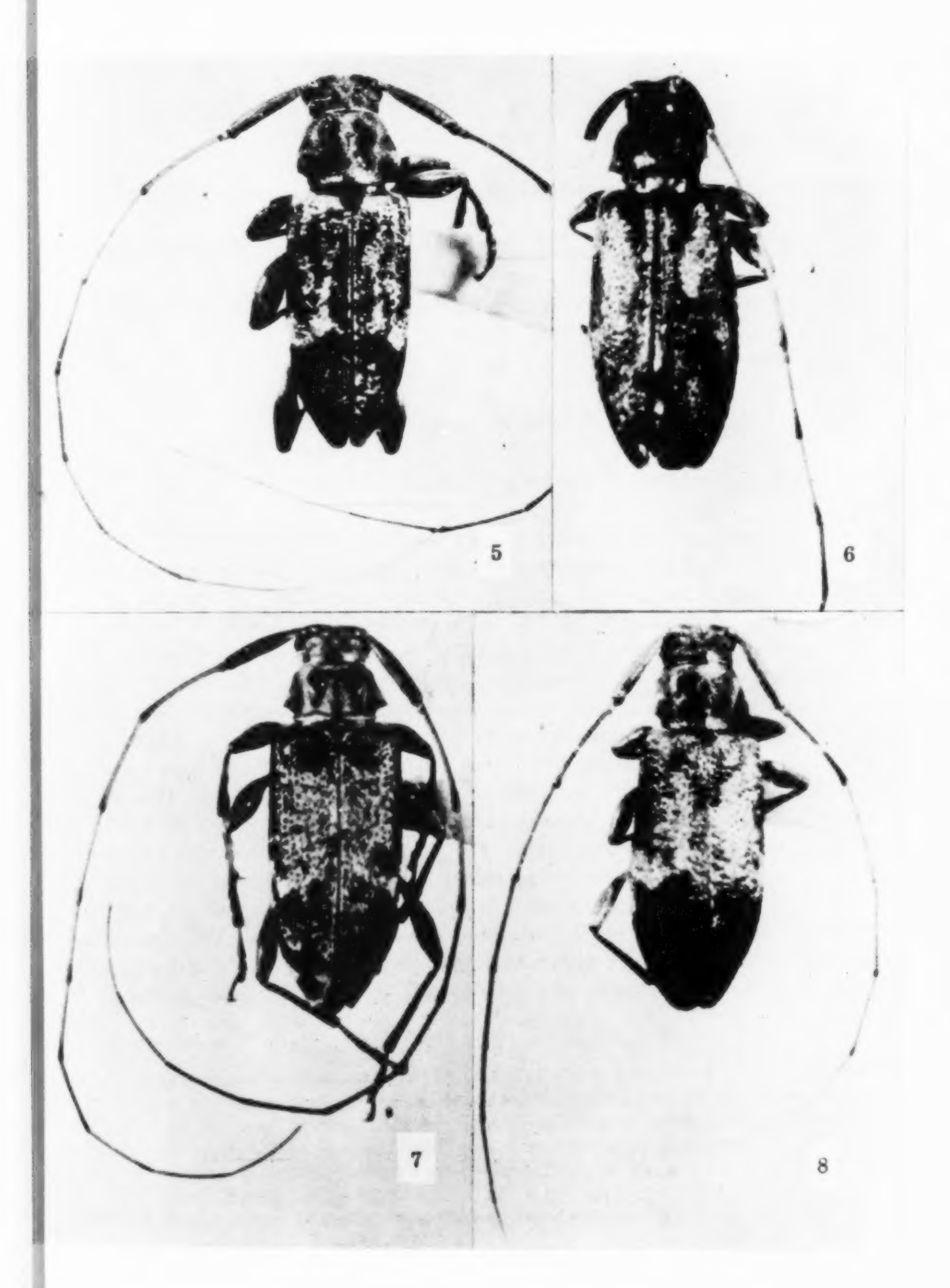

1
2
3
4

Fig. 5: *Urgleptes vauricarnum* sp. nov. ♂. Holotype.

Fig. 6: *U. sinnosa* sp. nov. ♀. Holotype.

Fig. 7: *U. bimaculata* sp. nov. ♀. Allotype.

Fig. 8: *U. bicolorata* sp. nov. ♀. Holotype.


5
6
7
8



Rev. Biol. Trop., 8 (1): 25-33, 1960

Comparative physiological studies on four species of hemoflagellates in culture. III. Effect of the Krebs' cycle intermediates on the respiration*

by

Rodrigo Zeledón**

(Received for publication February 8, 1960)

The functioning of a Krebs' cycle in trypanosomes was investigated with negative results in *Trypanosoma evansi* by MARSHALL (14) and in *T. hippicum* by HARVEY (9). MOULDER (16) investigating the problem in the bloodstream form of *T. lewisi* concluded that, since addition of some of the intermediate compounds did not enhance the respiration rate of the flagellates, the tricarboxylic acid cycle is not active in these organisms. BAERNSTEIN (4) studied the malic dehydrogenase and found a fumarase and a fumaric hydrogenase in homogenates of the culture form of *Schizotrypanum cruzi*. SEAMAN (19) demonstrated succinic dehydrogenase activity in homogenates of *S. cruzi* and AGOSIN and VON BRAND (1) have studied this enzyme, which is linked to the mitochondrial fraction, in the same organism. The plant parasite, *Strigomonas oncopelti* (=*Phytomonas sp.* ?) seems to have several of the dehydrogenases of the cycle (18). VON BRAND and AGOSIN (5), in manometric experiments, demonstrated the oxidation of some of the intermediates of the cycle in the culture forms of *Leishmania tropica* and *S. cruzi* using whole organisms. Furthermore, these authors reported that the strong inhibition produced by malonate is reversed by succinate. Similar work was done by MEDINA *et al.* (15)

---

* This paper is based on a portion of a dissertation submitted in partial fulfillment of the requirements for the degree of Sc. D. in the School of Hygiene and Public Health, Johns Hopkins University, during the tenure of a John Simon Guggenheim Fellowship. This work was carried out at the Department of Pathobiology, School of Hygiene and Public Health, and supported in part by a grant from the U. S. Public Health Service (E-783).

** Departamento de Parasitología, Universidad de Costa Rica.

using the culture form of *L. brasiliensis*. Finally AGOSIN and WEINBACH (2) have characterized a TPN-linked isocitric dehydrogenase in *S. cruzi*. The present report deals with experiments on the oxidation of Krebs' cycle intermediates by the culture forms of four species of hemoflagellates. Appreciation is expressed to Dr. Clark P. Read for his interest in this work and kind advice and criticism.

MATERIALS AND METHODS

ORGANISMS. *Endotrypanum schaudinni, Leishmania enriettii, Schizotrypanum cruzi* and *S. vespertilionis*. Details of the origin of the strains and of the culture medium used, have been given in a previous paper (22).

MANOMETRIC EXPERIMENTS. The organisms were harvested in the exponential phase of growth, washed by three repeated centrifugations in Krebs-Ringer phosphate (KRP), pH 7.2, and resuspended in an adequate volume of the same solution before use in manometric experiments or, in one series of experiments, in KRP at pH 4.5 (final pH 5.0). Oxygen consumption was determined by conventional Warburg respirometry using duplicate flasks for each substrate which, after addition, attained a final concentration of 0.01 M. Control vessels containing glucose were included in each experiment and endogenous respiration was recorded in two control flasks. The experiments were conducted under atmospheric air, at a temperature of 30° C, and oxygen uptake was followed for 2 hours. Details of the procedures have been already described (22). The intermediates, including pyruvate and acetate, were used as the sodium salt or the acid form neutralized with NaOH. Isocitric acid was prepared from the lactone form by hydrolysis in dilute NaOH according to the method of KREBS and EGGLESTON (12).

NITROGEN DETERMINATIONS. These were carried out either by the technique of KABAT and MAYER (11) or by the nesslerization procedure described by LANG (13). In both cases, the same standard solution of ammonium sulfate was used.

RESULTS

The results of the effect of acetate, pyruvate and the Krebs' cycle intermediates at two different pH's are presented in Tables 1 and 2. In none of the cases were the stimulations so clear cut at pH 7.2 as at the lower pH. At the higher pH, the stimulations varied, in general, between 10 and 40 per cent above the endogenous respiration and, in a few cases, some of the tricarboxylic acids depressed the respiration to values below those of endogenous controls. In one case (*L. enriettii*) acetate inhibited endogenous respiration and the phenomenon was true regardless of the pH. At pH 5.0 pronounced stimulation of respiration was observed upon addition of Krebs' cycle intermediates; in *E. schaudinni*, alpha-ketoglutarate, malate, and pyruvate yielded essentially the same respiratory rate as glucose. Succinate in the same species gave a stimulation

TABLE 1

*Effect of Krebs' cycle intermediates on respiration at pH 7.2. Each value represents the **mean** of one duplicate determination, unless otherwise stated. Glucose and endogenous values are given for comparison*

SPECIES		Endogenous	Glucose	Citrate	cis-Aconitate	iso-Citrate	alpha-Ketoglutarate	Succinate	Fumarate	Malate	Oxaloacetate	Pyruvate	Acetate
E. schaudinni	S/E	**1.00**	**13.80***	0.93	0.68	0.88	1.80	1.89	1.15	1.33	1.17	3.63	1.34
	S/G	0.09*	**1.00**	0.07	0.13	0.07	0.12	0.35	0.21	0.09	0.22	0.28	0.19
L. enriettii	S/E	**1.00**	7.18*	1.09	1.29	0.66	1.22	1.57	1.14	1.22	1.29	1.00	0.62
	S/G	**0.14***	1.00	0.21	0.15	0.07	0.19	0.19	0.14	0.19	0.15	0.19	0.08
S. vespertilionis	S/E	**1.00**	**3.04**	1.15	1.00	1.00	1.27	1.12	1.00	1.44	1.02	1.00	0.99
	S/G	0.36	**1.00**	0.36	0.49	0.49	0.42	0.53	0.48	0.48	0.49	0.31	0.39
S. cruzi	S/E	1.00	2.78*	1.03	0.93	1.04	1.04	1.25	1.00	1.15	1.02	0.94	0.97
	S/G	0.39*	**1.00**	0.46	0.43	0.51	0.48	0.58	0.46	0.53	0.47	0.42	0.45

S/E=ratio $Q^{N}O_2$ substrate/ $Q^{N}O_2$ endogenous; S/G=ratio $Q^{N}O_2$ substrate/ $Q^{N}O_2$ glucose $Q^{N}O_2 = \mu l$ of O_2/mg. N/hr.

* **Mean of 15 duplicate determinations.**

TABLE 2

Effect of Krebs' cycle intermediates on respiration at pH 5.0. Each value represents the mean of one duplicate determination, unless otherwise stated. Glucose and endogenous values are given for comparison .

SPECIES		Endogenous	Glucose	Citrate	cis-Aconitate	iso-Citrate	alpha-Ketoglutarate	Succinate	Fumarate	Malate	Oxaloacetate	Pyruvate	Acetate
E. schaudinni	S/E	1.00	9.65*	1.12	0.95	0.68	12.19	14.50	8.95	12.04	2.14	8.08	0.58
	S/G	0.10*	1.00	0.25	0.11	0.06	0.96	1.35	0.83	0.95	0.19	0.95	0.07
L. enriettii	S/E	1.00	4.93*	0.90	1.25	1.07	1.91	1.71	1.36	1.55	1.13	1.25	0.59
	S/G	0.20*	1.00	0.22	0.24	0.19	0.36	0.31	0.25	0.39	0.21	0.24	0.14
S. vespertilionis	S/E	1.00	2.70*	1.20	1.03	1.09	1.77	1.60	1.13	1.83	1.10	1.22	0.69
	S/G	0.37*	1.00	0.51	0.44	0.47	0.58	0.70	0.49	0.60	0.48	0.52	0.30
S. cruzi	S/E	1.00	2.56*	1.12	0.96	1.12	2.04	1.60	1.16	1.66	1.06	1.43	0.64
	S/G	0.39*	1.00	0.42	0.35	0.42	0.76	0.58	0.42	0.60	0.38	0.53	0.24

S/E and S/G ratios as in Table 1.
* Mean of 2 duplicate determinations.

35 per cent higher than that of the glucose control at the same low pH. In *L. enriettii* the values for respiratory stimulation are only slightly higher at the low pH, and for the two *Schizotrypanum* they were significantly higher in some instances. *L. enriettii, S. vespertilionis* and *S. cruzi* utilized pyruvate only at pH 5.0 at a very low rate. At the same low pH, acetate seemed to be toxic for all four species. The increase in respiration produced by the compounds at the low pH was quite stable. Examples are illustrated in figures 1, 2 and 3.

DISCUSSION

The present observations on the utilization of Krebs' cycle intermediates by four species of hemoflagellates indicate that the external pH is an important factor in the detection of their oxidation. The influence of low pH can be understood in terms of the ionization of the intermediates. It is known that the unionized molecules of weak electrolytes penetrate cells by diffusion more readily than the ions (10). This account for the differences observed at the two different pH's. It may also be postulated that the lower pH will modify the permeability properties of the cell membrane making the substrate available to the enzymes. Furthermore, the data suggest that the tricarboxylic acids penetrate the cell with great difficulty; this is in agreement with the results obtained by VON BRAND and AGOSIN (5) in the case of *S. cruzi* and *L. tropica*. The latter authors observed somewhat more utilization of the tricarboxylic acids by *S. cruzi* in isotonic KCl at pH 5.6.

The vigorous utilization of pyruvate by *E. schaudinni,* with evidence of a very active Krebs' cycle in this species, suggests that glucose might be oxidized completely, or nearly so, by this parasite. The low rate of oxidation of pyruvate by *L. enriettii* and the two *Schizotrypanum* is consistent with the observation that the compound is only partially oxidized by the flagellates. Changes in utilization rate of the compound in cultures, accompanied by physiological and morphological changes in the case of *S. cruzi* (7), await further investigation. A diphasic growth curve has been reported in cultures of the latter species (6, 8). We agree with VON BRAND and AGOSIN (5) that a fundamental change in metabolic pattern of the organisms is not necessarily responsible for the second peak shown by *S. cruzi*. As the pH drops as a consequence of carbohydrate fermentation, the organisms are able to metabolize pyruvic and succinic acids (two important products first accumulated in the medium), but it is not known whether the organisms of the second growth curve utilize these products at a higher rate than those belonging to the first growth curve.

The data presented here suggest that the complete tricarboxylic acid cycle is present in the four organisms studied, although it might have a somewhat different physiological function in each particular case. The possibility that only a part of the cycle may operate is apparent. BAERNSTEIN (3) could not demonstrate aconitase in the culture form of *S. cruzi* and has presented (4), for the same organism, the scheme of a partial cycle with a very active malic dehydrogenase coupled to a fumarase and a fumaric hydrogenase, giving rise

to succinate. The system, at the same time, would be connected in some way to the cytochromes, presumably through flavoproteins. On the other hand, the recent demonstration of isocitric dehydrogenase in *S. cruzi* by AGOSIN and WEINBACH (2) and the sensitivity of the flagellate to fluoroacetate (21), seem to support the occurrence of the complete cycle.

It may be that in *Schizotrypanum* and *Leishmania* the tricarboxylic acid cycle plays a minor role in energy production but supplies the precursors of a series of amino acids, as seems to be the case in *Escherichia coli* (17) and in *Mycobacterium butyricum* (20). We believe that, at least for *Schizotrypanum,* transminating enzymes may play a very important role in connection with this phase of energy metabolism. As far as *E. schaudinni* is concerned, the Krebs' cycle is probably quite important as a pathway for pyruvate oxidation and consequent energy production; the inhibition by fluorecetate with a corresponding decrease in motility seems to support this idea (21). Likewise, the high rate of succinate oxidation by this organism suggests the importance of the succinoxidase system.

SUMMARY

The effect of the Krebs' cycle intermediates upon respiration of the culture forms of *Endotrypanum schaudinni, Leishmania enriettii, Schizotrypanum vespertilionis* and *S. cruzi* was examined at two different hydrogen ion concentrations. At pH 7.2, low stimulations with some of the intermediates were observed. At pH 5.0 the stimulations in general were more evident, and alpha-ketoglutarate, malate, and pyruvate were oxidized by *E. schaudinni* at the same rate as glucose while succinate produced a respiratory stimulation 35 per cent higher than that of glucose. At the same low pH, the other three species were able to utilize some pyruvate, whereas acetate inhibited all of them. The increase in respiration produced by the tricarboxylic acids was almost negligible, regardless of the pH, and in some instances the substances rather had a depressive action on the respiration. It is believed that the tricarboxylic acid cycle is present in the four species and the possibility of its importance in roles other than energy production, in *Leishmania* and *Schizotrypanum,* is discussed.

RESUMEN

Se estudia el efecto de las sustancias intermediarias del ciclo de Krebs en la respiración de las formas de cultivo de *Endotrypanum schaudinni, Leishmania enriettii, Schizotrypanum vespertilionis* y *S. cruzi,* a dos diferentes concentraciones de iones de hidrógeno. A pH 7.2 se observaron estímulos pequeños; a pH 5.0 los estímulos respiratorios fueron más evidentes y en el caso de *E. schaudinni,* alfa-cetoglutarato, malato y piruvato fueron oxidados con la misma intensidad que glucosa y el succinato produjo un estímulo respiratorio 35 por ciento más elevado que el producido por el carbohidrato. Al mismo pH

ácido, las otras tres especies fueron capaces de utilizar piruvato, mientras que el acetato fue inhibitorio para todas. Los ácidos tricarboxílicos produjeron un aumento insignificante a cualquiera de los dos pH y en algunos casos hubo un efecto depresivo sobre la respiración. Se concluye que el ciclo de Krebs existe en las cuatro especies y se discute la posibilidad de que el mismo tenga importancia en otros aspectos metabólicos, que no sean la producción de energía, en el caso de *Leishmania* y *Schizotrypanum.*

REFERENCES

1. AGOSÍN, M., & T. VON BRAND
1955. Characterization and intracellular distribution of the succinic dehydrogenase of *Trypanosoma cruzi. Exp. Parasitol.,* 4: 548-563.

2. AGOSÍN, M., & E. C. WEINBACH
1956. Partial purification and characterization of the isocitric dehydrogenase from *Trypanosoma cruzi. Biochim. et Biophys. Acta,* 21: 117-126.

3. BAERNSTEIN, H. D.
1953. The enzyme systems of the culture form of *Trypanosoma cruzi. Ann. N. Y. Acad. Sci.,* 56: 982-994.

4. BAERNSTEIN, H. D.
1953. Malic dehydrogenase and related enzymes in the culture form of *Trypanosoma cruzi. Exp. Parasitol.,* 2: 380-396.

5. BRAND, T. VON & M. AGOSÍN
1955. The utilization of Krebs cycle intermediates by the culture forms of *Trypanosoma cruzi* and *Leishmania tropica. J. Infect. Dis.,* 97: 274-279.

6. CHANG, S. L.
1947. Studies on hemoflagellates. I. A semisolid medium and a fluid medium with a solid base for growing various species of *Leishmania* and *Trypanosoma cruzi. J. Infect. Dis.,* 80: 164-171.

7. CHANG, S. L.
1948. Studies on hemoflagellates IV. Observations concerning some biochemical activities in culture, and respiration of three species of *Leishmania* and *Trypanosoma cruzi. J. Infect. Dis.,* 82: 109-118.

8. CHANG, S. L. & W. O. NEGHERBON
1947. Studies on hemoflagellates II. A study of the growth rates of *Leishmania donovani, L. brasiliensis, L. tropica* and *Trypanosoma cruzi* in culture. *J. Infect. Dis.,* 80: 172-184.

9. HARVEY, S. C.
1949. The carbohydrate metabolism of *Trypanosoma hippicum. J. Biol. Chem.,* 179: 435-453.

10. JAMES, W. O.
1953. The use of respiratory inhibitors. *Ann. Rev, Plant Physiol.,* 4: 59-90.

11. KABAT, E. A., & M. M. MAYER
1948. *Experimental Immunochemistry*, 567 pp. Charles C. Thomas, Illinois.

12. KREBS, H. A., & L. V. EGGLESTON
1944. Micro-determination of iso-citric and cis-aconitic acids in biological material. *Biochem. J.*, 38: 426-437.

13. LANG, C. A.
1958. Simple microdetermination of Kjeldahl nitrogen in biological materials. *Anal. Chem.*, 30: 1692-1694.

14. MARSHALL, P. B.
1948. The glucose metabolism of *Trypanosoma cruzi* and the action of trypanocides. *Brit. J. Pharmacol.*, 3: 8-14.

15. MEDINA, H., D. AMARAL, & M. BACILA
1955. Estudos sobre o metabolismo de protozoarios do gênero *Leishmania*. I. Vias de oxidação da glicose e do acetato pela *Leishmania brasiliensis* Vianna, 1911. *Arq. Biol. Tecnol.*, 10: 97-102.

16. MOULDER, J. W.
1948. The oxidative metabolism of *Trypanosoma lewisi* in a phosphate saline medium. *J. Infect. Dis.*, 83: 33-41.

17. ROBERTS, R. B., D. B. COWIE, R. BRITTEN, E. BOLTON, & P. H. ABELSON
1953. The role of the tricarboxylic acid cycle in amino acid synthesis in *Escherichia coli*. *Proc. Natl. Acad. Sci.*, 39: 1013-1019.

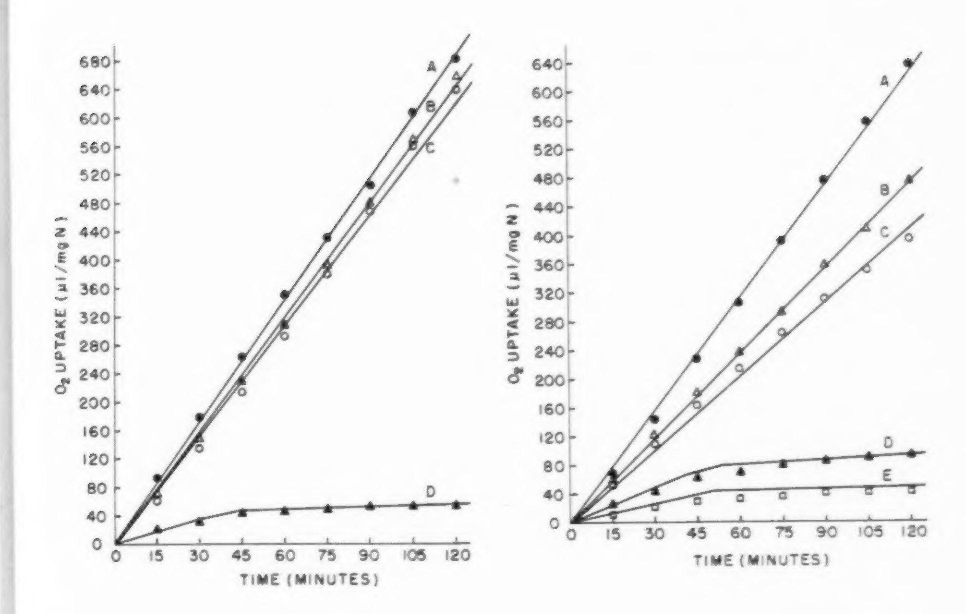
18. RYLEY, J. F.
1955. Studies on the metabolism of the Protozoa. 4. Metabolism of the parasitic flagellate *Strigomonas oncopelti*. *Biochem. J.*, 59: 353-361.

19. SEAMAN, G. R.
1953. The succinic dehydrogenase of *Trypanosoma cruzi*. *Exp. Parasitol.*, 2: 236-241.

20. WRIGHT, D. E.
1959. The formation of keto and amino acids by *Mycobacterium butyricum* growing in a chemically defined medium. *J. Gen. Microbiol.*, 20: 554-565.

21. ZELEDÓN, R.
1959. Unpublished data.

22. ZELEDÓN, R.
1960. Comparative physiological studies on four species of hemoflagellates in culture. I. Endogenous respiration and respiration in the presence of glucose. *J. Protozool.* 7: 146-150.



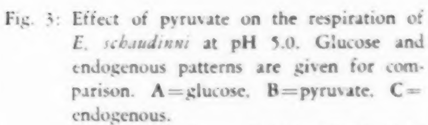
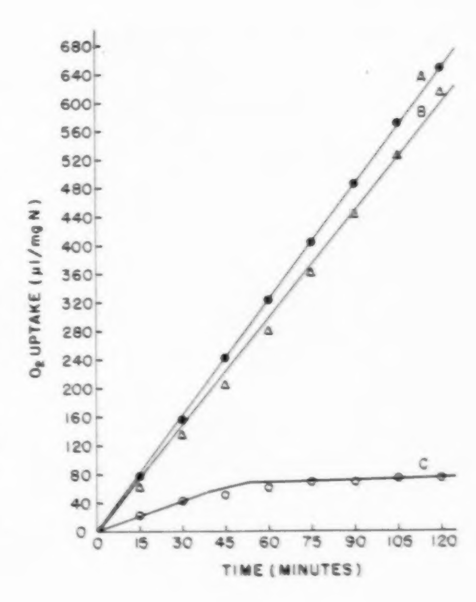


Fig. 1: Effect of Krebs' cycle intermediates on respiration of *E. schaudinni* at pH 5.0. Glucose and endogenous patterns are given for comparison. A=glucose. B=alpha-ketogluturate. C=malate. D= endogenous.

Fig. 2: Effect of Krebs' cycle intermediates on respiration of *E. schaudinni* at pH 5.0. Glucose and endogenous patterns are given for comparison. A=succinate. B=glucose. C = fumarate, D=oxaloacetate. E=endogenous.

Fig. 3: Effect of pyruvate on the respiration of *E. schaudinni* at pH 5.0. Glucose and endogenous patterns are given for comparison. A=glucose. B=pyruvate. C=endogenous.





Rev. Biol. Trop., 8 (1): 35-47, 1960

The subgenera of Culicoides of the Americas (Diptera, Ceratopogonidae)

by

L. Vargas*

(Received for publication February 15, 1960)

The genus *Culicoides* comprises a large number of species of wide distribution. As more species become known, and our knowledge of their morphology, ecology and life habits increases their natural affinities and interrelationships become clearer to us. The present study is an attempt to define the subgenera of *Culicoides* present in the American continent; since most of them have a world-wide distribution, a completely satisfactory understanding of their status, definition and composition will require contributions from investigators in many countries. Not all the subgenera that have been proposed are discussed here, nor is an attempt made to place every known species of *Culicoides* in a certain subgenus. There are many valid names of species that can be so identified, but not to the subgeneric level. We have tried to relate wing and male genitalia characters; there seems to be a good correlation between them, and the subgenera proposed may prove generally acceptable, although more knowledge, especially of early stages of development, is necessary for a more critical evaluation of the present treatment.

SUBGENERA OF *CULICOIDES* OF THE AMERICAS DISCUSSED IN THIS PAPER, WITH TYPES

Subgenus	Type
Anilomyia n. subgen.	*covagarciai*
Avaritia Fox, 1955	*obsoletus*
Beltranmyia Vargas, 1953	*crepuscularis*
Culicoides s. str.	*pulicaris*
Diphaomyia n. subgen.	*baueri*
Drymodesmia n. subgen.	*copiosus*
Glaphiromyia n. subgen.	*scopus*
Haemotomyidium Goeldi, 1905	*paraensis*
Hoffmania Fox, 1947	*insignis*
Macfiella Fox, 1955	*phlebotomus*
Mataemyia n. subgen.	*mojingaensis*
Monoculicoides Khalaf, 1954	*nubeculosus*
Oecacta Poey, 1851	*furens*
Selfia Khalaf, 1954	*hieroglyphicus*

* Instituto de Salubridad y Enfermedades Tropicales, México.

KEY TO THE SUBGENERA OF *CULICOIDES* USING CHARACTERS OF THE MALE GENITALIA

1 — Mesosome cross-shaped and with and added, curved process, longitudinally parallel to the longest arm of the cross .... *Macfiella*
— Mesosome entirely different 2
2 — Aedeagus "bottle- shaped"; ventral root of basistyle absent or small; parameres with sharp points, sometimes with very small terminal hairs 3
— Aedeagus different; ventral root of basistyle absent or present; paramers with points bare, or spinose, or with subapical lobe, or with small hairs 4
3 — Parameres with strongly angulated base with point directed laterally, never fusioned; distal margin of the ninth tergum with a median notch and well developed apicolateral processes; inner side of basistyle without short pilosity .... *Anilomyia* n. subgen.
— Parameres with a common base or this expanded; distal margin of ninth tergum commonly convex, with a median notch and apicolateral processes small, usually convergent; inner side of basistyle usually with short pilosity .... *Hoffmania*
4 — Basistyle usually without ventral root or this very short; inner side with short pilosity. Parameres strongly angulated at the base, with point directed laterally; tips usually with short hairs. Distal margin of ninth tergum usually convex and with short apicolateral processes .... *Culicoides* s. str.
— Basistyle with ventral root well developed; inner side without short pilosity. The parameres if strongly angulated do not have terminal hairs. Distal margin of ninth tergum with or without median notch; with or without long apicolateral processes 5
5 — Parameres fused in a single piece 6
— Parameres never fused 7
6 — Parameres bifid; aedeagus prominently bifid .... *Monoculicoides*
— Parameres not bifid; aedeagus not bifid .... *Selfia*
7 — Basistyle with ventral root well developed, without notch; parameres without terminal spines, subapical lobe or other processes, at most with fine terminal hairs 8
— Basistyle with ventral root notched or with a retrose tooth; parameres with subapical lobe or spinose tip or both 12
8 — Bases of the parameres strongly angulate, points directed laterally 9
— Bases of parameres not strongly angulate, only slightly ondulated; aedeagus usually arch-shaped 10
9 — Distal margin of ninth tergum convex and without apicolateral processes or these very small; aedeagus Y-shaped .... *Avaritia*
— Distal margin of ninth tergum truncated, with apicolateral processes directed inwards .... *Drymodesmia* n. subgen.
10 — Bases of parameres are broad and their sharp tips are directed proximally .... *Beltranmyia*
— Bases of parameres not greatly expanded and without sharp tips 11
11 — Parameres with distal half with curves .... *Glaphiromyia* n. subgen.
— Parameres with only a distal curve .... *Mataemyia* n. subgen.
12 — Parameres with subapical lobe 13
13 — Aedeagus Y-shaped, truncated, with small flaps at the sides of the central stem .... *Oecacta*
— Aedeagus usually arch-shaped, distally is rounded .... *Haematomyidium*

KEY TO DETERMINE SUBGENERA OF *CULICOIDES* USING FEMALE EXTERNAL CHARACTERS

1 — Wings dark, without pale markings .... *Selfia*
— Wings different 2
2 — Wing spots not very marked; machrotrichia sparse; small specimens .... *Avaritia*
— Wings with very apparent markings 3
3 — Second radial cell of the wing included in a pale spot 4
— Second radial cell of the wing included in a dark spot 5
4 — Cell R_5 of the wing with a marginal dark spot shaped like a sand-glass; without pale spot on vein M_2 .... *Culicoides* s. str.
— Cell R_5 of the wing without dark spot shaped like a sand-glass; pale spot on vein M_2 .... *Hoffmania, Anilomyia* n. subgen.
5 — The fourth segment of the hind tarsus is not cordiform 6
— The fourth segment of the hind tarsus is not cordiform 6
6 — With pale oval spot on vein M_1 and M_2; pale oval spot subterminal on cell M_2 .... *Glaphiromyia* n. subgen.
— With a pale spot on M_1, or on M_2, or without spots on these veins 7
7 — Usually with a pale oval spot on vein M_2 of the wing........ *Diphaomyia* n. subgen.
— Usually the vein M_2 of the wing without pale oval spot........ 8
8 — Usually the cross-vein r-m is dark .... *Haematomyidium*
— The cross-vein r-m is not dark 9
9 — From the second radial cell to the tip of the wing, on cell R_5, usually there are only two pale spots; mesonotum usually without dark dotting 10
— From the second radial cell to the tip of the wing, on cell R_5, usually there are more than two pale spots 12
10 — Veins M_1 and M_2 of the wing within long pale bands 11
— Vein M_1 and M_2 never included in pale bands .... *Beltranmyia*
11 — Usually there is an oval pale spot on vein M_1 of the wing .... *Drymodesmyia* n. subgen.
— Usually without pale spot on vein M_1 of the wing .... *Mataemyia* n. subgen.
12 — Veins C_1 and C_2 of the wing included in broad pale bands .... *Monoculicoides*
— Veins C_1 and C_2 of the wing not included in broad pale bands or they are very narrow .... *Oecacta*

Anilomyia n. subgen.
subgenus type *covagarciai* Ortiz, 1950

rostratus Wirth & Blanton, 1956.
cockerellii (Coquillet, 1901).
luteovenus Root & Hoffman, 1937.

Wirth and Blanton (1959) mention three groups in the subgenus *Culicoides* Latreille s. str.: *pulicaris, covagarciai* and *nigrigenus*. The *covagarciai* group is here treated and presented as a new subgenus.

Diagnosis: Long to medium-sized specimens. Legs yellow or with femoral tibial joint broadly yellow-banded; mesonotum yellowish to brown, subshining, with few markings. Third segment of the palpus slender or the last segment about half as long as the third segment or longer. *Anilomyia* n.

subgen. differs from the group *nigrigenus* in that the palpus of the latter has the third segment much swollen and is over twice as long as the last segment.

Wings light-colored with small dark markings. The darkest spot occurs basal to the second radial cell. This cell wholly or mostly included in a light spot.

Male genitalia: As in the *pulicaris* group, the inner margins of the basistyles are spinose. The distal margin of the ninth tergum may have well developed apicolateral processes or not. Ventral root of the basistyle absent or marked only by a small point. The aedeagus is "bottle-shaped", as in the subgenus *Hoffmania*. Parameres fused or closely appressed, with a terminal brush.

Female genitalia: Two spermathecae well developed.

In the species *pulicaris*, the subgenus type of *Culicoides* s. str., the parameres are very much like those of *Anilomyia* n. subgen. but the aedeagus is Y-shaped. The mesonotum in *Anilomyia* n. subgen. shows very few markings; this is in sharp contrast with *pulicaris*.

The dark spot on the second radial cell present in the subgenus *Beltranmyia*, or the absence of markings, or the slight spots on the wing, as are seen in *Selfia*, *Oecacta* or *Monoculicoides*, are good characters to separate these subgenera from *Anilomyia* n. subgen.

DISTRIBUTION: Nearctic and Neotropical regions.

Beltranmyia Vargas, 1953
Subgenus type *crepuscularis* Malloch, 1915

travisi Vargas, 1949
nanus Root & Hoffman, 1937
wirthi Foote & Pratt, 1954
alaskaensis Wirth, 1951
antefurcatus Wirth & Blanton, 1959
crescentis Wirth & Blanton, 1959
daedalus Mackie, 1948
commatis Wirth & Blanton, 1959
pampoikilus Macfie, 1948
phaeonotus Wirth & Blanton, 1959
lutealaris Wirth & Blanton, 1956
daedaloides Wirth & Blanton, 1959
chrysonotus Wirth & Blanton, 1956
knowltoni Beck, 1946
bermudensis Williams, 1956

WIRTH & BLANTON (1959) pointed out that a number of species, which they called the *daedalus* group, was quite homogeneous and distinct from others included in the subgenus *Oecacta*, whose type is *furens*. In our opinion the

species of the *daedalus* group are Panamenian species of *Beltranmyia* and therefore are very close to the nearctic *crepuscularis* (Malloch, 1915), *canithorax* Hoffman, 1925, *salinarius* Kieffer, 1914, *travisi* Vargas and others.

NEW DEFINITION. Medium-sized specimens. Third segment of the palpus moderately swollen. Fourth and fifth segments together over half as long as the third segment. The fourth segment as long as broad. Thorax usually with many markings. Wing dark with pale markings with many long hairs; distal part of the first radial cell and all the second radial cell covered by the darkest spot of the wing. A pale spot on cross vein r-m and the proximal half of the first radial cell. No dark spot is included in this pale area.

Male genitalia: Ninth tergum with well marked apicolateral processes but of size variable from one species to another. Distal margin with median notch. The ventral root of the basistyle slender, usually short and smaller than the dorsal root, never as long as in the *iriartei* group, without the retrograde process. Dististyle hook-shaped. Aedeagus V- or Y-shaped with truncated, broad tip. Parameres never bent at right angles, as in *pilosus, poikilonotus* or *jamaicensis*. Parameres with pointed proximal end. The ninth sternum is a narrow band.

Female genitalia: Two big spermathecae. One in *crepuscularis*.

Culicoides s. str.

DISTRIBUTION: Nearctic and Neotropical regions.

Subgenus type *pulicaris* Linnaeus, 1758

neopulicaris Wirth, 1955
tristiatulus Hoffman, 1925
yukonensis Hoffman, 1925

DIAGNOSIS: Mesonotum not uniformly marked. Never with many, small round dots. Third segment of the palpus only slightly engrossed. Wing with the darkest spot covering the distal half of the first radial cell and the proximal half of the second radial cell. A light spot that reaches the costa covers the transverse vein r-m. Cell R_5 with very large light areas, at the middle there is a dark spot in the shape of a sandglass. Cell M_1 with two large light areas. Cell M_2 with apical light spot that reaches the margin. Cell M_4 with a central light spot.

Male genitalia: Basistyle with the inner margin covered with short but strong pilosity; with or without a ventral root. Parameres with sharply bent bases close together, the tips bearing short hairs.

Aedeagus V-shaped. Ninth sternum very high. Ninth tergum with convergent apicolateral processes and without median notch on the distal margin.

Female genitalia: Two spermathecae.

DISTRIBUTION: Mostly nearctic with some extension to the northern limit of the neotropical region.

Diphaomyia n. subgen.

Subgenus type *baueri* Hoffman, 1925

iriartei Fox, 1952
evansi Wirth & Blanton, 1959
haematopotus Malloch, 1915
footei Wirth, 1956
blantoni Vargas & Wirth, 1955

DIAGNOSIS: The species of this subgenus were formerly considered as included in the subgenus *Oecacta* Poey. Mesonotum with several markings. Distal half of the first radial cell and all the second radial cell included in a dark spot. Cell R_5 with several pale spots. A well marked light spot on vein M_1. Other light spot on M_2. More or less round, light spots on cells M_1 and M_2.

Male genitalia: Ventral root of the basistyle long, curved, and rounded apically, with a very small notch at the base. Parameres with small bases, long, slender and apically with many short spines. Aedeagus with short processes on the arch, near the main stem. Distal margin of the ninth tergum convex and usually with small apicolateral processes.

Female genitalia: Two spermathecae.

DISTRIBUTION: Nearctic and Neotropical.

The black spot on the second radial cell separates *Diphaomyia* n. subgen. from *Avaritia, Hoffmania, Culicoides* s. str., and *Anilomyia*. The light spots on veins M_1 and M_2 recall those of *Glaphiromyia*, the other subgenus with this character, but are very easily distinguished because in *Diphaomyia* n. subgen. the parameres have many spines, and are neither plain nor adorned with very small terminal hairs. The ventral root of the basistyle is notched at the base, the apicolateral processes of the ninth tergum are neither very prominent nor broad-based. The arch of the aedeagus in *Glaphiromyia* lacks the small submedian processes.

Drymodesmyia n. subgen.

Subgenus type *copiosus* Root & Hoffman, 1937

jamaicensis Edwards, 1922
loughnani Edwards, 1922
panamensis Barbosa, 1947
antunesi Forattini, 1954
copiosus Root & Hoffman, 1937 (n. syn. *pilosus* W. & B. 1959)
biguttatus (Coquillett, 1901)
poikilonotus Macfie, 1948
dunni Wirth & Blanton, 1959
mulrenani Beck, 1957
wirthomyia Vargas, 1953.

DIAGNOSIS: Small specimens. Third palpus segment greatly swollen. Mesonotum usually with several markings. Wing hairy. A dark spot covers the distal half of the first radial cell and the whole of the second radial cell. The cross vein r-m included in a pale spot. Cell R_5 usually with one pale spot, not

strangled, contiguous to the second radial cell and another pale spot about the middle of the cell R_5. Sometimes the vein M_2 divides a clear spot.

Male genitalia: Ninth tergum with well developed apicolateral processes. The middle notch not well marked. Basistyle with ventral hook of medium size, never with retrorse hook. Dististyle hook-shaped. Aedeagus with long and broad main body tip with sharp transverse cut. Parameres with long, sharp tips devoid of spines or hairs. The base bent about 90 degrees, broadened.

Female genitalia: Two large spermathecae.

C. dunni might belong here but as the male is unknown no strong statement is advanced.

The shortest central stem in the species of the subgenus *Beltranmyia* and the less angulated parameres differentiate the male genitalia of species included in *Drymodesmia* n. subgen.

From the so called *iriartei* and other groups differs because in these the parameres are not strongly angulate, bear several prominent spines and the ventral hook of the basistyle is either too long or has a retrose spine. This late character occurs in *furens* and related species of the subgenus *Oecacta*. Also the parameres show a globose process at the joint of the spinose tips.

C. wirthmoyia is very close to or identical with, *jamaicensis* but there are doubts as some *jamaicensis* have clear spots on the wing different from *wirthomyia* and the apicolateral processes of the ninth tergum are very short (see WIRTH, 2, p. 113, fig. 3). Also in *wirthomyia* the second radial cell is about half as long as the first radial cell. This characters induced FORATTINI (1) to consider the species as *Dasyhelea*.

DISTRIBUTION: Nearctic and Neotropical regions.

Glaphiromyia n. subgen.
Subgenus type *scopus* Root & Hoffman, 1937

villosipennis Root & Hoffman, 1937
dampfi Root & Hoffman, 1937
pecosensis Wirth, 1955
guttipennis (Coquillett, 1901)
arboricola Root & Hoffman, 1937
bakeri Vargas, 1954

DIAGNOSIS: Medium-sized or large specimens. Mesonotum usually with many markings. Wings with the distal half of the first radial cell and all the second cell included in a dark spot. Light markings on each of veins M_1 and M_2. The cross vein r-m, which is within a light spot, is dark in some species.

Male genitalia: Long and sharp ventral root of the basistyle, without retrorse tooth or notch. Parameres long, with curved base and blunt apex pointing basally. The tips are slender and show small terminal hairs. Aedeagus in the shape of an ample arch and a long stem. Apicolateral processes of the ninth tergum well prominent, set on a broad base.

DISTRIBUTION: Nearctic and Neotropical.

Thorax without the punctiform dots so characteristic of the *furens* groups. The ventral root of basistyle unnotched and without a retrorse tooth differentiates *Glaphiromyia* n. subgen. from the *furens* groups of *Oecacta* Poey. The parameres lack the globose subapical process or the long terminal spines of the *furens* groups of *Oecacta* or *Haematomyidium*. The main stem of the aedeagus lacks the small blades present in the *furens* groups of *Oecacta*. For other distinctions see under *Diphaomyia*.

Haemotomyidium Goeldi, 1905
Subgenus type *paraensis* (Goeldi, 1905)

lopesi Barreto, 1944
leopoldoi Ortiz, 1951
horticola Lutz, 1913
camposi Ortiz & León, 1955
carpenteri Wirth & Blanton, 1953
galindoi Wirth & Blanton, 1953
dicrourus Wirth & Blanton, 1955
carsiomelas Wirth & Blanton, 1955
alabialinus Barbosa, 1952
limai Barretto, 1944
stellifer (Coquillett, 1901)
debilipalpis Lutz, 1913

pachymerus Lutz, 1914
stigmalis Wirth, 1952
lanei Ortiz, 1950
balsapambensis Ortiz & León, 1955
castillae Fox, 1946
propiipennis Macfie, 1948
tetrahyris Wirth & Blanton, 1959

DIAGNOSIS: No uniformity of characters is seen in the mesonotum wing markings or palpi joints. The only constant distinctions are the dark spot covering the second radial cell and distal half of the first radial cell, and the immediately basal clear spot that embraces the transverse r-m vein. There are various other clear spots either on cells or veins.

Male genitalia: Basistyle with the ventral well developed and with a retrorse tooth. Root shape variable. Parameres without a broadened base; with subapical, globose processes and spinose tips. Aedeagus arched and with a prominent main stem rounded apically. Apicolateral processes of the ninth tergum usually well developed.

DISTRIBUTION: Few nearctic representatives, mostly neotropical.

In *Haematomyidium* Goeldi we include most of the species usually placed in *Oecacta* Poey. As the type of *Oecacta*, the species *furens*, has so definite

characters of a group we think that it is more convenient to limit the definition of *Oecacta* to a very homogenous, if small, number of species and open *Haematomyidium* for the multitude of species more or less related to *paraensis*.

The criterium to separate subgenera according to the number of spermathecae, one or two, is not a sound one, on an absolute basis, as that would separate for example two closely related species *castillae*, with one spermatheca, from *balsapambensis* and *spurius* with two spermathecae.

Hoffmania Fox, 1947
Subgenus type *insignis* Lutz, 1913

foxi Ortiz, 1950
hylas Macfie, 1940
flavivenula Lutz, 1937
guttatus (Coquillett, 1904)
ignaciosi Forattini, 1957
lutzi Lima, 1937
marium Lutz, 1913
ruizi Forattini, 1954
travassosi Forattini, 1957
venustus Hoffman, 1925

Diagnosis: Mesonotum usually marked in a uniform pattern. A wide central pale band in which the prescutellar spot shows very strongly. The darkest spot of the wing covers all or only the distal half of the first radial cell and a small portion of the second radial cell. The cross vein r-m dark, within a large light spot. Beyond the first radial cell usually there are only two large light spots. Cell M_1 usually with one light spot. Vein M_2 with one light spot. Veins M_1 and M_2 may be light all along. Cell M_2 with one apical light spot. Branches of the medio-cubital fork usually white all along.

Male genitalia: Basistyle without ventral root or this slightly marked. Aedeagus "bottle-shaped" as in *Anilomyia* n. subgen. Parameres with a very large base and tapering apically, with or without a small brush. The base of the parameres is fused in some species. The ninth tergum with small apicolateral processes pointing toward the mid-line.

Female genitalia: two spermathecae.

Distribution: Nearctic and Neotropical region but mostly in the latter.

Subgenus type *mojigaensis* Wirth & Blanton, 1953
Mataemyia n. subgen.

azureus Wirth & Blanton, 1959
lyrionotatus Wirth & Blanton, 1955
caprilesi Fox, 1952

DIAGNOSIS: Medium-sized specimens. Mesonotum usually with several round light areas in a dark background. Wing: A black spot covers the apical half of the first radial cell and all or most of the second radial cell. The second radial cell too long in *caprilesi*. The cross-vein r-m is included in a light area. Cell R_5 with two large clear spots of irregular shape. Veins M_1 and M_2 in light bands. Cell M_1 with two large light areas. Cell M_2 with one marginal, oval light area.

Male genitalia: Basistyle with prominent ventral root. This root without a retrorse tooth or a notch. Parameres long, with sharp points, without spines or small hairs. The base of the parameres is not directed basally. Aedeagus triangular in shape. Ninth tergum with well developed apicolateral processes and median notch.

Female genitalia: Two spermathecae.

DISTRIBUTION: Neotropical.

The darkest spot on the wing on second radial cell separates *Mataemyia* n. subgenus from genera like *Culicoides* s. str., *Anilomyia* n. subgen. and *Hoffmania*.

The absence of oval light areas on veins M_1 and M_2 separates this new subgenus from *Glaphiromyia* n. subgen. The absence of parameres with spines or hairs or the absence of a subapical globose process separates *Mataemyia* n. subgen. from *Oecacta*. *Haematomyidium* Goeldi stands apart by the retrorse tooth of the ventral root.

Oecacta Poey, 1851
Subgenus type *furens* (Poey, 1851)

venezuelensis **Ortiz & Mirsa, 1950**
barbosai **Wirth & Blanton, 1956**
gorgasi **Wirth & Blanton, 1953**

Oecacta Poey is a homogeneous subgenus which includes few species. Most of the heterogeneous groups now form other subgenera. *Haematomyidium* Goeldi is proposed to include the more closely related species. *Oecacta* is distinguished by the pattern of numerous punctiform dots on the mesonotum; third segment of the palpus not very engrossed; by the dark spot on the second radial cell and apical half of the first radial cell and by the three clear spots on cell M_1 with absence of clear spot on vein M_2.

Male genitalia: Basistyle with the ventral root well developed, with a retrorse tooth. Parameres with small base, with subapical globose process and spinose tips. Aedeagus triangular in shape, the tip is short, finely serrated, flanked by lateral flaps. Usually well developed apicolateral processes on the ninth tergum.

Female genitalia: Two spermathecae.

DISTRIBUTION: Nearctic and Neotropical.

SUMMARY

The subgenera of *Culicoides* are discussed using as examples species from the Americas. A clear understanding should be achieved when these species are compared with those from outside the Americas. Proposed as new subgenera are *Anilomyia, Diphaomyia, Drymodesmia, Glaphiromyia* and *Mataemyia*. Type species and related species are mentioned.

RESUMEN

Se discuten los subgéneros de *Culicoides* usando como ejemplo especies de América. Un estudio más profundo se logrará cuando se cotejen especies de otros lugares. Se proponen como nuevos subgéneros *Anilomyia, Diphaomyia, Drymodesmia, Glaphiromyia* y *Mataemyia*. Las especies tipos y las cercanas se mencionan.

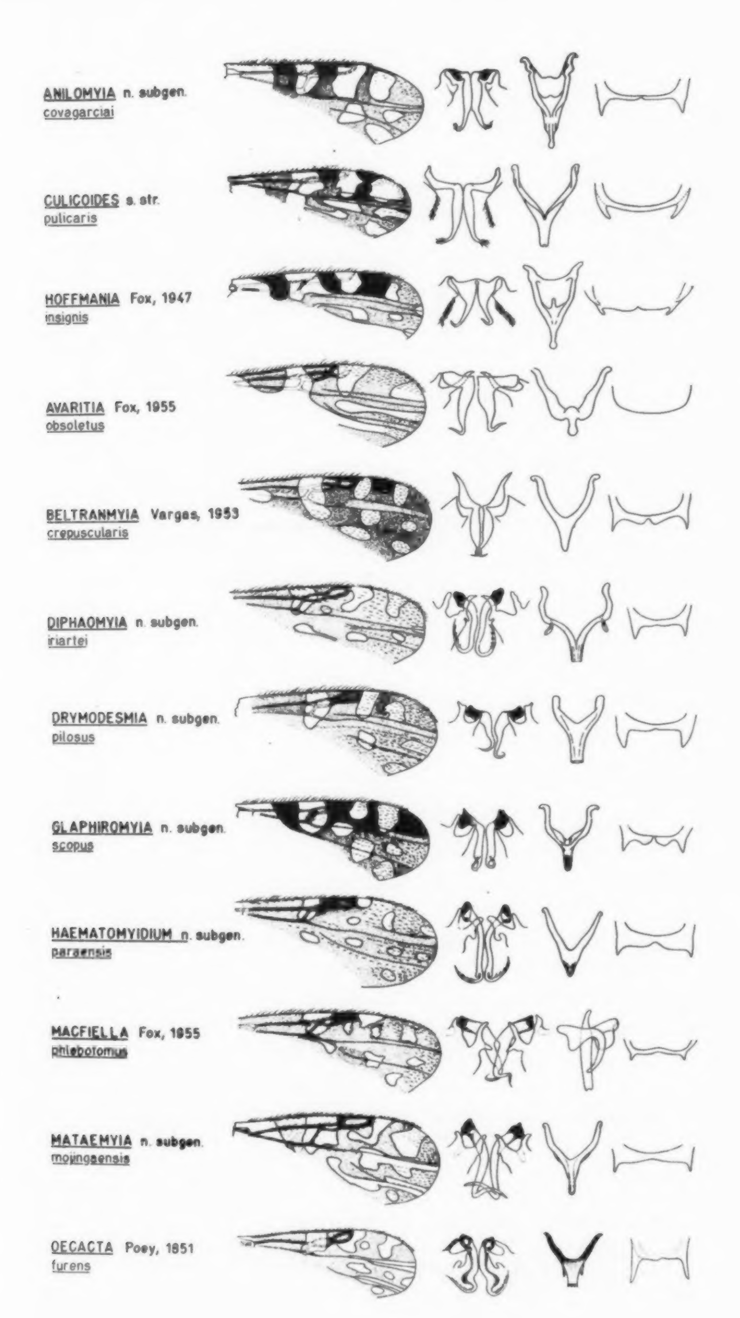
REFERENCES

1. FORATTINI, O. P.
   1957. *Culicoides* da Região Neotropical (Diptera, Ceratopogonidae). *Arq. Fac. Hig. Saúde Publ. Univ. São Paulo*, 11 (2): 160-526.

2. WIRTH, W. W.
   1955. Report on a collection of biting midges of the genus *Culicoides* from Guatemala. *Proc. Ent. Soc. Wash.*, 57 (3): 109-117.

3. WIRTH, W. W. & F. S. BLANTON
   1959. Biting midges of the genus *Culicoides* from Panamá (Diptera: Heleidae). *Proc. U. S. Nat. Mus.*, 109 (3415): 237-482.

Fig. 1: Morphological characteristics of the subgenera of *Culicoides*.


ANILOMYIA n. subgen.
covagarciai
CULICOIDES s. str.
pulicaris
HOFFMANIA Fox, 1947
insignis
AVARITIA Fox, 1955
obsoletus
BELTRANMYIA Vargas, 1953
crepuscularis
DIPHAOMYIA n. subgen.
iriartei
DRYMODESMIA n. subgen.
pilosus
GLAPHIROMYIA n. subgen.
scopus
HAEMATOMYIDIUM n. subgen.
paraensis
MACFIELLA Fox, 1955
phlebotomus
MATAEMYIA n. subgen.
mojingaensis
OECACTA Poey, 1851
furens



Rev. Biol. Trop., 8 (1): 49-52, 1960

Salmonella en bovinos adultos, aparentemente sanos, destinados al consumo

por

Guido Arroyo* y Róger Bolaños**

(Recibido para su publicación el 1º de marzo de 1960)

Durante el año de 1955 se presentó una epizootia de etiología desconocida en bovinos embarcados con destino a Curazao. Los médicos veterinarios sospecharon que podría tratarse de una salmonelosis provocada por una disminución de la resistencia de los animales a consecuencia de la fatiga, hacinamiento y alimentación deficiente durante el transporte. Aunque esta situación no deja de ser hipotética, creímos que sería interesante establecer algunos de los tipos de *Salmonella* que pueden encontrarse en bovinos aparentemente sanos. En esta forma pensamos dar un aporte a la epidemiología de la salmonelosis, tanto bovina como humana.

MATERIAL Y METODOS

En el Matadero Municipal de San José se recogieron muestras de materia fecal, trozos de intestino, ganglios mesentéricos, bazo, hígado o, en su lugar, una porción de bilis, provenientes de cada uno de 195 animales destinados al consumo.

Los órganos se quemaron en su superficie con espátula caliente, tomándose luego 2-3 gramos de su interior para hacer un macerado que sirviera de inóculo; del fragmento de intestino se practicó un raspado de la mucosa; las materias fecales y la bilis fueron trabajadas sin tratamiento previo alguno. Las siembras de los diferentes materiales se realizaron en los medios de enriquecimiento y aislamiento primario para *Salmonella*, según recomendaciones clásicas (3). Las colonias sospechosas fueron identificadas tanto bioquímica como serológicamente.

---

* Laboratorio de Investigaciones Médico Veterinarias, Ministerio de Agricultura e Industrias, San José, Costa Rica.

** Departamento de Microbiología, Universidad de Costa Rica.

RESULTADOS

Al examen macroscópico las muestras empleadas no mostraron, en ningún caso, signos de algún proceso infeccioso activo.

Veintiséis animales dieron cultivos positivos por salmonelas, obteniéndose así una incidencia total de 13,3 por ciento. De ellos, veinte presentaron únicamente un tipo de muestra positivo, mientras que de los seis restantes uno presentó tres y el resto dos; en todos los casos donde más de una muestra permitió aislamientos positivos, siempre fue encontrado el mismo serotipo por animal.

Las mayores frecuencias de aislamientos las aportaron la mucosa intestinal (11 veces) y los ganglios mesentéricos (10 veces), siguiéndoles el bazo con seis cultivos y por último el hígado (o la bilis) y las materias fecales, con tres. Las diferencias observadas entre el número de aislamientos obtenidos a partir de la mucosa intestinal y los de la materia fecal son estadísticamente significativos ($p<0,05$). Por esta razón, creemos que las muestras de heces fecales no son adecuadas para investigar portadores bovinos de *Salmonella*, en animales con condiciones semejantes a las de los nuestros.

De los veintiséis animales positivos logramos aislar veintisiete diferentes cepas, al encontrar dos salmonelas diferentes en el cultivo hecho a partir del raspado de la mucosa intestinal de uno de ellos (*S. newport* y *S. muenchen*). Estas cepas fueron identificadas como *S. panama* (8 veces), *S. newport* (8 veces), *S. give* (4 veces), *S. abortus-bovis* (2 veces), *S. london* (2 veces), *S. thyphi-murium* (1 vez), *S. muenchen* (1 vez) y *S. edinburg* (1 vez).

DISCUSION

Nos ha llamado la atención el hecho de que la muestra que menos cultivos nos aportó fue la de materia fecal, contrastando así con los resultados de MONTEVERDE y sus colaboradores (5) en Argentina, en los cuales se observa que precisamente de este material obtuvieron un número elevado de aislamientos. Además, nuestros mejores resultados fueron obtenidos con ganglios mesentéricos y mucosa intestinal, mientras que SIMEONE (7), también en Argentina y con vacunos que presumimos sean de condiciones similares a los usados por Monteverde, encuentra una incidencia sumamente baja de salmonelas en los ganglios mesentéricos. Un resultado similar al de SIMEONE, en cuanto a ganglios se refiere, fue obtenido por NEIVA en Brasil (6). Por otro lado, los autores mexicanos (9) demuestran incidencias de diez por ciento o más de salmonelas en hígado y bazo de bovinos normales, situación que también viene a ser bastante diferente a la que hemos encontrado en nuestro medio.

FIELD (4) señala la posibilidad de que condiciones desfavorables predispongan al animal a la salmonelosis. Los nuestros son frecuentemente obligados a permanecer durante varios días en corrales poco adecuados, con alimentación deficiente o nula y, algunas veces, tienen que recorrer largas distancias. Es posible que las diferencias que hemos señalado en cuanto a la localización

anatómica de las salmonelas en las diferentes regiones del continente americano, puedan originarse en factores de este tipo. Es posible también, que sean ellos los que influyan en el aparecimiento de cuadros clínicos de salmonelosis en animales exportados a otros países y que la diferencia entre una y otra situación sea exclusivamente de tiempo. Así, en un brote que se presentó en ganado bovino exportado de Costa Rica a Aruba, cuidadosamente estudiado por los investigadores radicados en esa isla (8), se llegó a confirmar su etiología salmonelósica. Los serotipos aislados, con excepción de *S. oslo* y *S. havana*, han sido señalados en el territorio nacional y los porcentajes de mortalidad observados en esos casos (alrededor de veinte por ciento) se asemejan bastante a los de incidencia de salmonelas que hemos encontrado en nuestros bovinos aparentemente normales. De ahí que pensemos, como también lo hicieron los autores antes citados (8), que alguna causa durante el trasporte provocó el aparecimiento de la enfermedad y, además creemos, que ésta bien pudo ser de origen endógeno, en los animales que ya tenían el microorganismo.

Las cepas aisladas por nosotros no representan una gran variedad de serotipos, pero creemos que son de importancia por ser las primeras que se obtienen en el país de bovinos aparentemente normales. También señalamos por primera vez en el territorio nacional *S. abortus-bovis*.

Es importante recalcar que *S. dublin*, uno de los agentes más importantes en la patología veterinaria, no ha sido encontrado por nosotros, no sólo en bovinos aparentemente sanos, sino que tampoco en unos pocos casos de enteritis infecciosa de terneros que hemos tenido oportunidad de estudiar (1); asimismo, no aparece citada en ninguno de los estudios que han sido realizados en nuestro país y que fueron recopilados recientemente por BOLAÑOS (2).

AGRADECIMIENTO

Los autores desean dejar constancia de su gratitud al Dr. Gerardo Varela, del Instituto de Enfermedades Tropicales de México, por haber confirmado serológicamente las cepas aisladas; al personal del Instituto de Estadística de la Universidad de Costa Rica por el análisis de los datos y al señor Rodrigo Montoya, supervisor de carnes en el Matadero Municipal, por su desinteresada colaboración en la toma de muestras.

RESUMEN

Ciento noventa y cinco bovinos adultos, aparentemente sanos y destinados al sacrificio en el Matadero Municipal de San José, han sido estudiados con la intención de localizar e identificar organismos del género *Salmonella*. Fue demostrada una incidencia total de 13,3 por ciento a partir de muestras de heces fecales, mucosa intestinal, ganglios mesentéricos, bazo, hígado o bilis. La materia fecal proporcionó un porcentaje mínimo de cultivos positivos (3), mientras que la mucosa intestinal y los ganglios mesentéricos fueron los mejores materiales (11 y 10 respectivamente). En orden de frecuencia los serotipos encontrados fueron los siguientes: *S. panama, S. newport, S. give, S. abortus-*

bovis, *S. london*, *S. thyphi-murium*, *S. muenchen* y *S. edinburg*. Se hace un breve comentario de la importancia que pueden tener condiciones desfavorables al animal en relación a la obtención de bacterias de una determinada muestra. Asimismo, se comenta la relación que existe entre el embarque de ganado al exterior y los casos de salmonelosis.

SUMMARY

The occurrence of *Salmonella* was investigated in one hundred ninety-five adult, apparently healthy head of cattle brought to the Municipal Slaughterhouse of San Jose, Costa Rica. Material examined included feces, intestinal mucose, mesenteric ganglia, spleen, and liver or bile, with a total incidence of 13.3 per cent. Fecal matter gave a minimal number -3- of positive cultures, while intestinal mucose and mesenteric ganglia gave the highest yields -11 and 10, respectively. The serotypes found were, in order of frequency: *S. panama*, *S. newport*, *S. give*, *S. abortus-bovis*, *S. london*, *S. typhi-murium*, *S. muenchen* and *S. edinburg*.

Brief remarks are made on the effect of conditions adverse to the animals on the isolation of bacteria from the samples, and its relation to the outbreaks of salmonellosis among cattle for export.

BIBLIOGRAFIA

1. ARROYO, G. y R. BOLAÑOS
   1959. Enteritis infecciosa de los terneros. I. Estudio de tres brotes en diferentes localidades del país. *Rev. Biol. Trop.*, 7: 89-93.

2. BOLAÑOS, R.
   1958. Sobre la distribución e identificación serológica del género *Salmonella* en Costa Rica. *Rev. Biol. Trop.*, 6: 43-53.

3. EDWARDS, P. R. & H. W. EWING
   1955. *Identification of Enterobacteriaceae*. vii + 179 pp. Burges Publishing Co. Minneapolis, Minnesota.

4. FIELD, H. I.
   1949. *Salmonella* infection in cattle. *Vet. Rec.*, 61: 109-112.

5. MOOTEVERDE, J. J., D. H. SIMEONE y E. S. MENCHACA
   1946. Investigación de salmonelas en materias fecales de bovinos sanos. *Ann. 3° Congr. Bras. Vet., Porto Alegre*: 447-454.

6. NEIVA, C.
   1946. Salmonelas en ganglios dos bovinos. *Ann. 3° Congr. Bras. Vet., Porto Alegre*: 436-438.

7. SIMEONE, D. H.
   1947. Investigaciones de bacterias del género *Salmonella* en ganglios mesentéricos de bovinos normales. *Rev. Med. Cien. Afines*, 9: 9-15.

8. SUTMÖLLER, P. & E. H. KAMPELMACHER
   1957. The occurrence of *Salmonella* among animals in Aruba (Netherlands Antilles). *In. Trop. Prot. Dis. Vet. Fac. Utrecht*, 23: 207-217.

9. VARELA, G.
   1954. Microorganismos que suelen causar intoxicaciones por alimentos contaminados. *Pren. Med. Mex.*, 6: 1-4.

Rev. Biol. Trop., 8 (1): 53-61, 1960

Helmintos de la República de Costa Rica. XIV. Redescripción de Lutztrema obliquum Travassos, 1941, y descripción de Zonorchis macroovaricus n. sp.

por

Otto Jiménez-Quirós* y Guido Arroyo**

(Recibido para su publicación el 31 de marzo de 1960)

El material para estudio fue coleccionado por nosotros: un ejemplar de "oropéndola" (*Gymnostinops montezuma*) y otro de "cusinga" (*Pteroglossus torquatus torquatus*), clasificados por el Ing. Agr. Alfonso Jiménez, del Museo Nacional. En las vías biliares del primer ejemplar encontramos tres especímenes de *Lutztrema* de los cuales tan sólo uno nos permitió efectuar un estudio completo, y también en las vías biliares del segundo encontramos ocho de *Zonorchis* de los cuales tres nos permitieron realizar el estudio y descripción que presentamos. En ambos casos efectuamos preparaciones totales de los tremátodos, teñidas con carmín de Grenacher.

Familia.—DICROCOELIIDAE Odhner, 1911
Subfamilia.—Dicrocoeliinae Looss, 1899
Lutztrema obliquum (Travassos, 1917) Travassos, 1941.

Tremátodos de pequeña talla, de cuerpo fusiforme o claviforme muy alargado y con las extremidades atenuadas. Cutícula sin espinas y con papilas pequeñas dispuestas irregularmente. Mide 3,810 mm de largo por 0,396 mm de ancho al nivel de la zona acetabular. Ventosa oral sub-terminal que mide 0,133 mm de largo por 0,129 mm de ancho y que posee una pequeña

---

* Departamento de Parasitología, Facultad de Microbiología, Universidad de Costa Rica.
** Laboratorio de Investigaciones Médico-Veterinarias del Ministerio de Agricultura e Industrias, San José, Costa Rica.

prolongación del cuerpo, hacia adelante, en forma de labio. Acetábulo robusto y musculoso de mayor tamaño que la ventosa oral; mide 0,295 mm de largo por 0,295 mm de ancho. Relación entre las ventosas 1: 2,2. Faringe colocada inmediatamente debajo de la ventosa oral, relativamente fuerte y que mide 0,041 mm de largo por 0,029 mm de ancho. Esófago, que se continúa en ciego único, y que mide de la faringe al inicio de la bolsa del cirro 0,148 mm de largo por 0,029 mm de ancho. Ciego único sinuoso, que pasa detrás del acetábulo, visible parcialmente entre los testículos, éstos y el ovario, perdiendo su visibilidad detrás de las asas uterinas. Poro genital mediano, aproximadamente equidistante entre las ventosas. Bolsa del cirro bien desarrollada y que contiene el cirro, próstata y vesícula seminal. Mide 0,189 mm de largo por 0,078 mm de ancho. Testículos sub-triangulares de contornos ligeramente irregulares, situados de manera que coinciden parcialmente en los mismos campos y en la misma zona, presentando discreta oblicuidad recíproca. Miden, el anterior 0,198 mm de largo por 0,258 mm de ancho y el posterior 0,244 mm de largo por 0,281 mm de ancho. El ovario, de contorno regular, elipsoide, transversal, está situado debajo del testículo posterior. Mide 0,133 mm de largo por 0,161 mm de ancho. Glándula de Mehlis situada debajo del ovario, en posición central. Mide 0,050 mm de largo por 0,048 mm de ancho. Vitelinas laterales, constituídas por folículos relativamente voluminosos y en escaso número, situadas en el tercio medio del parásito. Se extienden: la izquierda 0,551 mm y la derecha 0,454 mm. Distan de la extremidad posterior: la izquierda 2,030 mm y la derecha 2,080 mm. El útero, bien desarrollado, ocupa toda el área del cuerpo debajo de las vitelinas y el área situada entre éstas. Tiene una rama descendente y otra ascendente, ésta última forma asas que bordean el ovario insinuándose entre éste y el testículo posterior, pasando luego entre ambos testículos y finalmente por el acetábulo, para terminar encima de éste en el poro genital. Huevos de color castaño oscuro, operculados, y que miden 0,034 mm de largo por 0,020 mm de ancho. Vesícula excretora no se observa en nuestros ejemplares.

HUÉSPED: *Gymnostinops montezuma* (Lesson) Sclater.

LOCALIZACIÓN: Conductos biliares.

DISTRIBUCIÓN GEOGRÁFICA: Chitaría, Peralta, Provincia de Cartago, Costa Rica.

EJEMPLARES: En la colección helmintológica del Laboratorio de Helmintología, Departamento de Parasitología, Facultad de Microbiología, Universidad de Costa Rica, bajo el No. 200-25.

DISCUSIÓN: Hemos clasificado los especímenes de Costa Rica con base en el trabajo de TRAVASSOS (3) como *Lutztrema obliquum*. Unicamente difieren de los ejemplares brasileños en algunos detalles mensurables, y el haber sido encontrados en un nuevo huésped.

Familia.—DICROCOELIIDAE Odhner, 1911
Subfamilia.—Dicrocoeliinae Looss, 1899
Zonorchis macroovaricus n. sp.

Tremátodos lanceolados y transparentes; miden 3,657 mm a 3,708 mm de largo por 0,523 mm a 0,762 mm de ancho a la altura del tercio medio. La cutícula está cubierta de pequeñas papilas cónicas, más evidentes en la extremidad anterior. El tegumento presenta numerosas escamas espiniformes, que van siendo menos numerosas hacia la extremidad posterior. La ventosa oral es sub-terminal y mide 0,261 mm a 0,290 mm de largo por 0,262 mm a 0,290 mm de ancho. La faringe, alargada y musculosa, mide 0,064 mm a 0,069 mm de largo por 0,092 mm a 0,106 mm de ancho. El esófago, más delgado que la faringe, mide 0,049 a 0,052 mm de ancho por 0,082 mm por 0,087 mm de largo. Los ciegos intestinales son de espesor similar al del esófago; en las ramas iniciales que rodean el acetábulo, se extienden, ligeramente flexuosos simétrica y lateralmente a lo largo del cuerpo; miden 0,050 mm a 0,053 mm de mayor ancho por 0,038 mm a 0,045 mm de menor ancho y se extienden. el derecho de 2,890 mm a 2,912 mm y el izquierdo de 2,749 mm a 2,762 mm. Las porciones terminales están casi totalmente cubiertas por las asas descendentes uterinas. El acetábulo, muy desarrollado y musculoso, más grande que la ventosa oral, está situado en el cuarto anterior del cuerpo; mide 0,395 mm a 0,399 mm de largo por 0,468 mm a 0,491 mm de ancho. Dista de la extremidad anterior de 0,578 mm a 0,584 mm. La relación de diámetros entre las ventosas es de 1: 1,5 a 1: 1,6.

El poro genital, subcircular, prebifurcal, está situado cerca de la porción distal de la faringe. La bolsa del cirro, oblonga y paraesofágica, mide de 0,166 mm a 0,170 mm de largo por 0,079 mm a 0,082 mm de ancho, y contiene vesícula seminal circunvoluta, próstata y cirro.

Los testículos, relativamente pequeños, masiformes y subesféricos, se encuentran en la misma zona, debajo del acetábulo, intracecales, pero separados entre sí por algunas asas uterinas. El testículo derecho mide 0,101 mm a 0,138 mm de diámetro ántero-posterior por 0,115 mm a 0,152 mm de diámetro transversal y el izquierdo mide 0,124 mm a 0,133 mm de diámetro ántero-posterior por 0,116 mm a 0,138 mm de diámetro transversal.

El ovario, grande y reniforme, situado en el lado derecho, coincide con el campo testicular del mismo lado. Mide 0,179 mm a 0,179 mm de diámetro antero-posterior por 0,225 mm a 0,247 mm de diámetro transversal. La glándula de Mehlis es elipsoidal y bien desarrollada, situada debajo del ovario, y mide 0,110 mm a 0,115 mm de diámetro ántero posterior por 0,082 mm a 0,142 mm de diámetro transversal.

Las glándulas vitelinas, localizadas en los campos laterales del cuerpo, en el tercio medio, están constituídas por numerosos folículos, en su mayoría extracecales. Toman inicio en la zona infratesticular para terminar muy antes de la finalización de los ciegos. Se extiende: la derecha 1,253 mm a 1,524 mm y la izquierda 1,391 mm a 1,473 mm.

El útero es muy desarrollado y está constituído por una rama ascendente y otra descendente, que con numerosas asas, en su mayoría intracecales, van desde la zona testicular hasta la región posterior del cuerpo. La rama descendente, particularmente desarrollada deja tan sólo un claro correspondiente a los ciegos que sobrepasan las vitelinas pero ocupan totalmente el cuarto posterior del cuerpo.

Los huevecillos, de cáscara gruesa, operculados y color castaño oscuro, miden 0,032 mm a 0,034 mm de largo por 0,020 mm a 0,027 mm de ancho.

La vesícula excretora no fue visible en nuestros ejemplares.

HUÉSPED: *Pteroglossus t. torquatus* (Gmelin).

LOCALIZACIÓN: Conductos biliares.

DISTRIBUCIÓN GEOGRÁFICA: El Silencio, Tilarán, Provincia de Guanacaste, Costa Rica.

EJEMPLARES: Holotipo y paratipos en la colección helmintológica del Laboratorio de Helmintología, Departamento de Parasitología, Facultad de Microbiología, Universidad de Costa Rica, bajo el No. 200-26.

DISCUSION

Según SKRJABIN (2) y YAMAGUTI (4), se conocen al presente 10 especies del género *Zonorchis* Travassos, 1944, que parasitan conductos y vesícula biliar de aves. BRENES y JIMÉNEZ (1) en 1959, describieron otra nueva especie *Z. costarricensis*.

Nuestros ejemplares han sido clasificados de acuerdo con la descripción original de TRAVASSOS (3) como pertnecientes al género *Zonorchis* y, con base en el estudio comparativo de nuestro material con las diagnosis específicas y figuras de las 11 especies hasta el presente descritas, hemos llegado a la conclusión de que se trata de una nueva especie (*Zonorchis macroovaricus*).

De las especies anteriormente descritas presenta tan sólo alguna analogía con *Z. angrense*, difiriendo de esa, sin embargo, por las siguientes características:

1. Mayor tamaño relativo y diferente forma del ovario (reniforme).
2. Diversa topografía de la glándula de Mehlis (en *Z. angrense* está situada hacia la línea mediana; en nuestros ejemplares hacia el campo lateral derecho).
3. Inicio y extensión de las vitelinas (en *Z. angrense* éstas se inician en la zona acetabular extendiéndose hasta la porción terminal de los ciegos; en nuestros ejemplares se inician en la zona infratesticular y terminan muy antes de la parte terminal de los ciegos).

RESUMEN

Se determina por primera vez para nuestro país, la presencia de *Lutztrema obliquum* (Travassos, 1917) Travassos, 1941, en *Gymnostinops monte-*

zuma (Icteridae), en la cual, hasta el presente, no se había reportado parasitismo por especies de *Lutztrema.*

Se describe una nueva especie, *Zonorchis macroovaricus,* parásito de vías biliares de "cusinga" *Pteroglossus torquatus torquatus* (Ramphastidae) en la que, hasta el presente, no se había reportado parasitismo por especies de *Zonorchis*

SUMMARY

Lutztrema obliquum (Travassos, 1917) Travassos, 1941, is reported for Costa Rica fort he first time, in the bile duct of *Gymnostinops montezuma* (Icteridae), in which family no previous record of *Lutztrema* parasitism has been published.

Zonorchis macroovaricus, n. sp. is described, from the bile duct of *Pteroglossus torquatus torquatus,* also the first record of *Zonorchis* parasitism in Rhamphastidae.

BIBLIOGRAFIA

1. BRENES, R. R. y O. JIMÉNEZ-QUIRÓS
1959. Helmintos de la República de Costa Rica XII. *Zonorchis costarricensis* n. sp. (Trematoda, Dicrocoeliidae) parásito de vías biliares de *Gymnostinops montezuma* (Icteridae). *Rev. Biol. Trop.,* 7 (1): 125-129.

2. SKRJABIN, K. I.
1952. *Trematody jivotnyj i cheloveka. Osnovy trematodologii.* VII. 762 pp. Isdatelsvo Akademii Nauk SSSR. Moskva.

3. TRAVASSOS, L.
1944. *Revisão da Familia Dicrocoeliidae Odhner, 1910.* Monografia do Instituto Oswaldo Cruz N° 2. 357 pp. + 124 pl.

4. YAMAGUTI, S.
1958. *Systema Helminthum.* I. (1). The digenetic trematodes of vertebrates. XI 979 pp. Interscience Publishers, Inc. New York.

Fig. 1: *Lutztrema obliquum* (Travassos, 1917) Travassos, 1941. Preparación total, vista ventral.

Fig. 2: *Lutztrema obliquum* (Travassos, 1917) Travassos, 1941. Extremidad anterior.

Fig. 3: *Lutztrema obliquum* (Travassos, 1917) Travassos, 1941. Huevos.

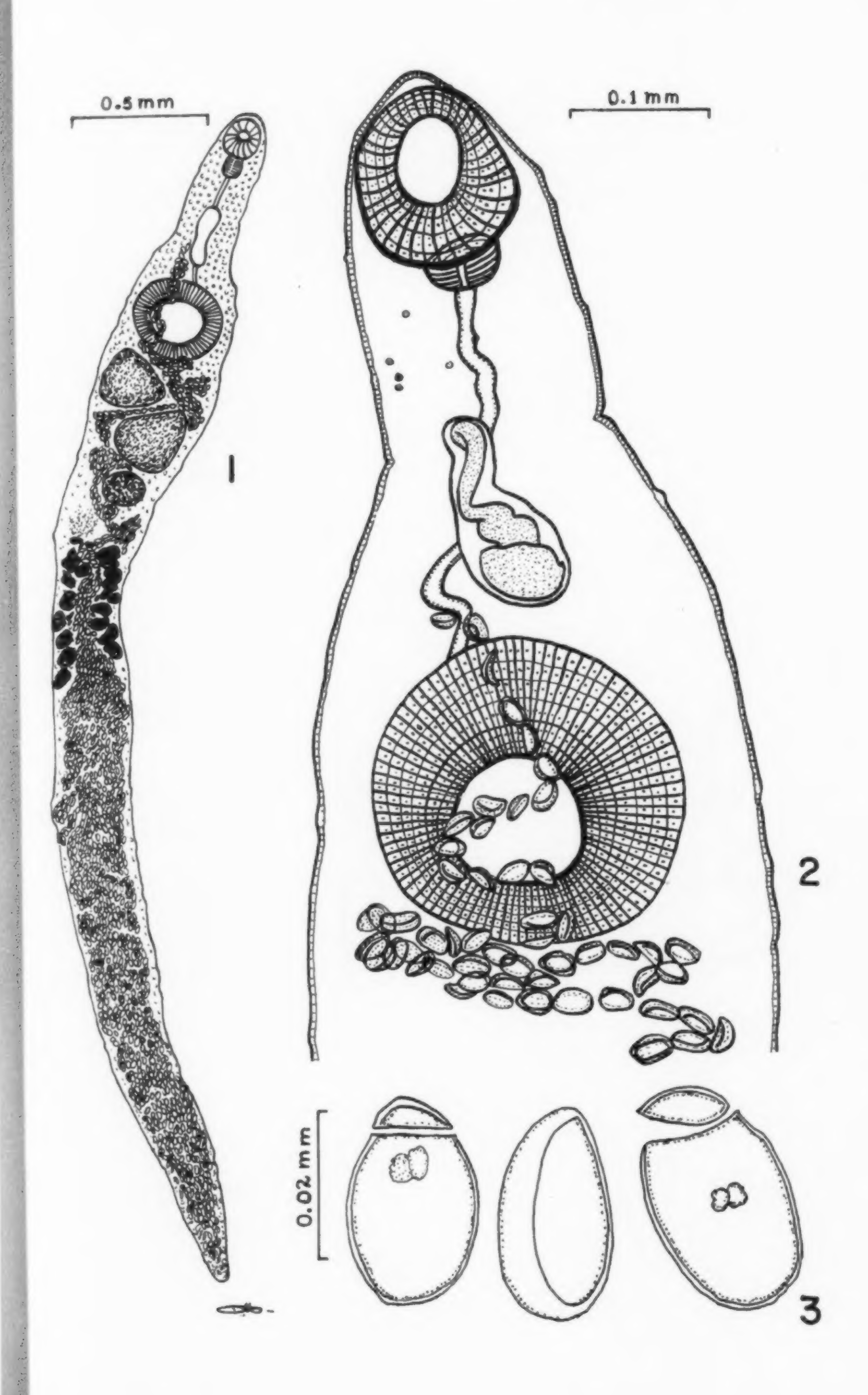
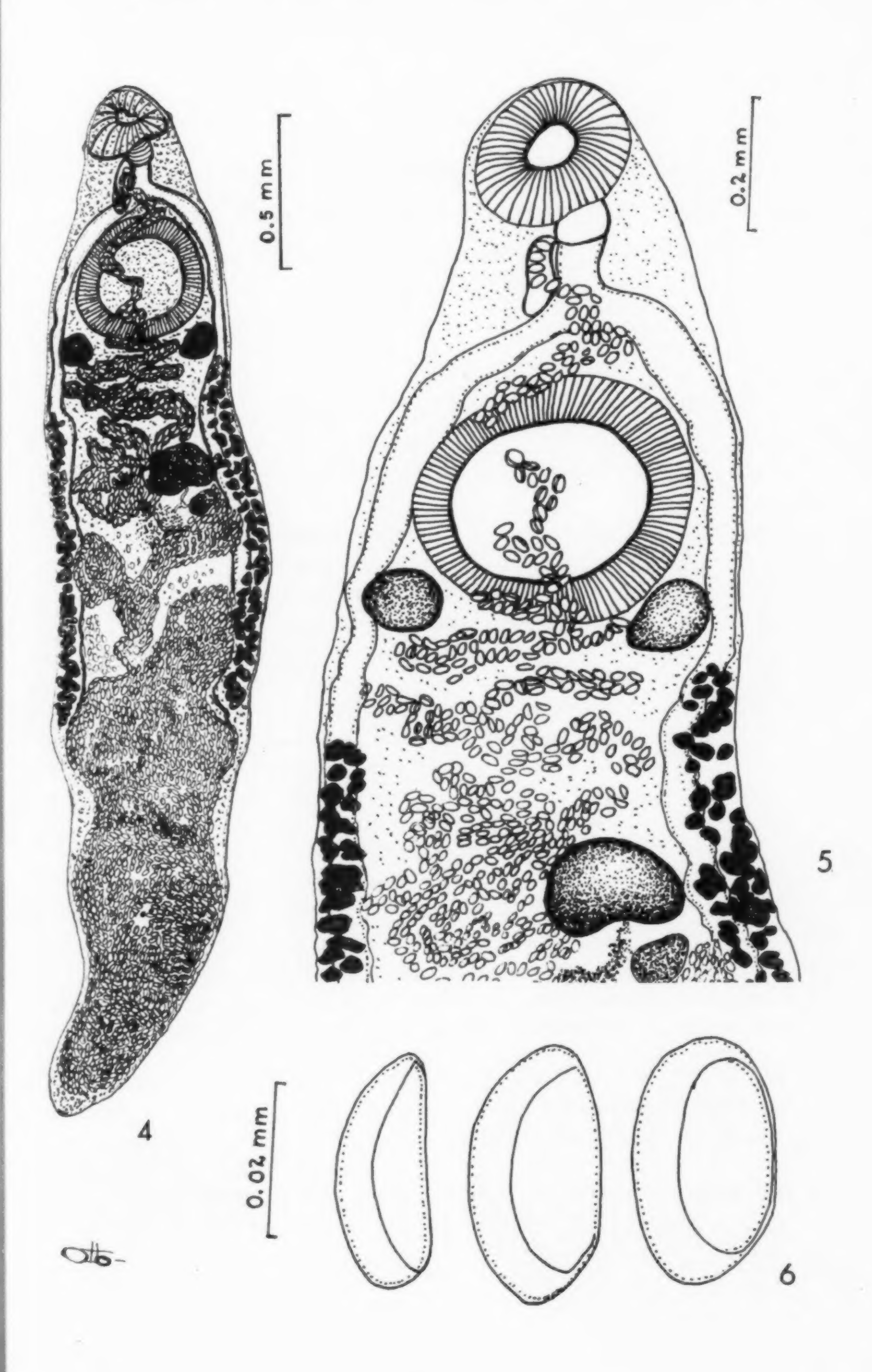

0.5 mm
1
0.1 mm
2
0.02 mm
3

Fig. 4: *Zonorchis macroovaricus* Jiménez & Arroyo, n. sp. Preparación total, vista ventral.

Fig. 5: *Zonorchis macroovaricus* Jiménez & Arroyo, n. sp. Extremidad anterior.

Fig. 6: *Zonorchis macroovaricus* Jiménez & Arroyo, n. sp. Huevos.


0.5 mm
0.2 mm
0.02 mm
4
5
6

N

la
co
pe
la

mu
vi
bra
su

do
en

---
*
**

Rev. Biol. Trop., 8 (1): 63-67, 1960

Helmintos de la República de Costa Rica. XV. Nematoda 3. Presencia de Litomosoides peñai n. sp. en Hemiderma perspicillatum aztecum (Saussure, 1860).

por

Otto Jiménez-Quirós* y Guido Arroyo**

(Recibido para su publicación el 31 de marzo de 1960)

Con el presente trabajo se da a conocer por primera vez en Costa Rica la presencia de un representante del género *Litomosoides* Chandler, 1931, encontrado en cavidad peritoneal de un murciélago y se describe una nueva especie: *L. peñai.* Las microfilarias encontradas por ZELEDÓN y VIETO (7) en la sangre del mismo huésped, posiblemente corresponden a esta especie.

Nuestro material de estudio lo obtuvimos de cavidad peritoneal de un murciélago (*Hemiderma perspicillatum aztecum*) capturado en Santa Ana, Provincia de San José, y consistió en numerosos ejemplares adultos machos y hembras que fueron fijados en alcohol-formalina y aclarados con lactofenol para su estudio e identificación.

Familia.—FILARIIDAE

Subfamilia.—Filariinae

Litomosoides peñai n. sp.*

Vermes filariformes, cilíndricos, de extremidad anterior ligeramente redondeada y extremidad distal cónica, siendo ésta en la hembra más regular y en el macho enrollada ligeramente en espiral. Cutícula muy finamente estriada

* Cátedra de Helmintología, Facultad de Microbiología, Universidad de Costa Rica.

** Laboratorio de Investigaciones Médico-Veterinarias del Ministerio de Agricultura e Industrias, San José, Costa Rica.

* La presente especie la dedicamos al Dr. Antonio Peña Chavarría, Decano de la Escuela de Medicina de la Universidad de Costa Rica.

excepto en las extremidades del cuerpo. Extremidad cefálica truncada cuando vista en posición dorsoventral y ligeramente redondeada vista de lado. Vestíbulo oral variable entre 0,028 y 0,035 mm con una luz vestibular cilíndrica y paredes densamente queratinizadas con protuberancias simétricas externas; de las últimas protuberancias las primeras fibras musculares del esófago toman inserción. Esófago muscular, no dividido en dos porciones, y que mide de 0,701 a 0,703 mm de largo; su decurso es regular, lo mismo su ancho a no ser en la extremidad proximal, donde toma relaciones con el vestíbulo oral, y se ensancha ligeramente a forma de bulbo. Hembras y machos presentan forma y tamaño similares.

El macho mide de 15,044 a 17,490 mm de largo con una anchura máxima en la extremidad cefálica de 0,044 mm y en la parte más ancha del cuerpo de 0,143 mm. El ancho máximo a la altura de la cloaca es de 0,033 mm. La cola, digitiforme, enrollada casi a círculo permite observar que la cloaca está situada en el cuarto distal a una distancia de 0,065 mm del ápice. Tres pares de papilas, número constante en todos los ejemplares, poco conspicuas y situadas en posición post-cloacal, distan a 0,027 mm, 0,038 mm y 0,050 mm, respectivamente del ápice caudal y el tercer par a 0,013 mm de la cloaca. Las espículas son desiguales y miden la pequeña de 0,055 a 0,059 mm de largo por 0,003 a 0,004 mm de ancho y la mayor de 0,180 a 0,199 mm de largo por 0,004 a 0,005 mm de ancho en sus respectivas partes quitinosas. La espícula mayor, en su parte filamentosa extracloacal mide 0,031 mm, siendo así de 0,168 mm la longitud máxima intracloacal de la espícula mayor. La relación entre espícula pequeña y mayor es de 1: 3,3.

La hembra mide de 71,214 a 76,590 mm de largo por un ancho máximo de 0,040 en la extremidad cefálica mediana y en la parte más ancha del cuerpo mide 0,227 mm. La vulva está situada a 0,726 mm de la extremidad anterior y en ese punto el ancho es de 0,122 mm. El ano está situado a 0,774 mm de la extremidad distal de la cola, la cual describe una pequeña curvatura regular y termina ligeramente redondeada, sin apéndices de ninguna especie; a la altura del ano la mayor anchura es de 0,113 mm.

HUÉSPED: *Hemiderma perspicillatum aztecum.*

LOCALIZACIÓN: Cavidad peritoneal.

DISTRIBUCIÓN GEOGRÁFICA: Santa Ana, Provincia de San José, C. R.

EJEMPLARES: 15 en la colección helmintológica del Laboratorio de Helmintología, Departamento de Parasitología, Facultad de Microbiología de la Universidad de Costa Rica, bajo el No. 3-4.

DISCUSION

Una vez revisada la literatura y comparadas las medidas y descripciones de los trabajos de CHANDLER (3), TRAVASSOS (5), ALMEIDA (1), OCHOTERENA y CABALLERO (4), VAZ (6) y CABALLERO (2), aunque nuestros ejemplares tengan parecido con *Litomosoides carinii* (Travassos, 1919), en vista de

una mayor profundidad del vestíbulo oral, una menor distancia entre vulva extremidad anterior, una menor distancia entre cloaca cola, una constante relación entre espícula menor y mayor de 1:3,3 y un número constante de papilas post-cloacales (tres pares), creemos que se trate de una nueva especie.

RESUMEN

Se señala por primera vez en Costa Rica, la presencia de un representante del género *Litomosoides* Chandler, 1931, y se describe una nueva especie *Litomosoides peñai*. Los ejemplares fueron obtenidos de cavidad peritoneal de un murciélago *Hemiderma perspicillatum aztecum* (Saussure, 1860).

SUMMARY

Litomosoides peñai n. sp. is described from several specimens from the peritoneal cavity of a bat *Hemiderma perspicillatum aztecum* (Saussure, 1890).

BIBLIOGRAFIA

1. Almeida, J. de Lins
   1936. Sobre un parásito de "CHEIROPTERA": *Litomosoides brasiliensis*, Rev. do Departamento Nacional da Producção Animal, 3 (1-6): 133-137.

2. Caballero, E.
   1944. Una nueva especie del género *Litomosoides* y consideraciones acerca de los caracteres sistemáticos de las especies de este género. *An. Inst. Biol. Mex.*, 15 (2): 383-388.

3. Chandler, A. C.
   1931. New genera and species of nematode worms. N° 2866. *Proc. U. S. Nat. Mus.* 78 (23): 23922-23931.

4. Ochoterena, I. y E. Caballero
   1932. Filaria parásita de las ratas de campo, *Micropleura sigmodoni* spec. nov. *An. Inst. Biol. Mex.*, 3 (2): 123-125.

5. Travassos, L.
   1919. *Filaria carinii* n. sp. *Rev. Soc. Bras. Sci.*, 3: 189.

6. Vaz, Z.
   1934. *Ackertia* gen. nov. for *Litomosa burgosi* de la Barrera, 1926, with notes on the synonymy and morphological variations of *Litomosoides carinii* (Travassos, 1919). *Ann. Trop. Med. Parasitol.*, 28 (2): 143-149.

7. Zeledón, R. y P. L. Vieto
   1957. Hallazgo de *Schizotrypanum vespertilionis* (Battaglia, 1904) en la sangre de murciélagos de Costa Rica. *Rev. Biol. Trop.*, 5 (2): 123-128.

excepto en las extremidades del cuerpo. Extremidad cefálica truncada cuando vista en posición dorsoventral y ligeramente redondeada vista de lado. Vestíbulo oral variable entre 0,028 y 0,035 mm con una luz vestibular cilíndrica y paredes densamente queratinizadas con protuberancias simétricas externas; de las últimas protuberancias las primeras fibras musculares del esófago toman inserción. Esófago muscular, no dividido en dos porciones, y que mide de 0,701 a 0,703 mm de largo; su decurso es regular, lo mismo su ancho a no ser en la extremidad proximal, donde toma relaciones con el vestíbulo oral, y se ensancha ligeramente a forma de bulbo. Hembras y machos presentan forma y tamaño similares.

El macho mide de 15,044 a 17,490 mm de largo con una anchura máxima en la extremidad cefálica de 0,044 mm y en la parte más ancha del cuerpo de 0,143 mm. El ancho máximo a la altura de la cloaca es de 0,033 mm. La cola, digitiforme, enrollada casi a círculo permite observar que la cloaca está situada en el cuarto distal a una distancia de 0,065 mm del ápice. Tres pares de papilas, número constante en todos los ejemplares, poco conspicuas y situadas en posición post-cloacal, distan a 0,027 mm, 0,038 mm y 0,050 mm, respectivamente del ápice caudal y el tercer par a 0,013 mm de la cloaca. Las espículas son desiguales y miden la pequeña de 0,055 a 0,059 mm de largo por 0,003 a 0,004 mm de ancho y la mayor de 0,180 a 0,199 mm de largo por 0,004 a 0,005 mm de ancho en sus respectivas partes quitinosas. La espícula mayor, en su parte filamentosa extracloacal mide 0,031 mm, siendo así de 0,168 mm la longitud máxima intracloacal de la espícula mayor. La relación entre espícula pequeña y mayor es de 1: 3,3.

La hembra mide de 71,214 a 76,590 mm de largo por un ancho máximo de 0,040 en la extremidad cefálica mediana y en la parte más ancha del cuerpo mide 0,227 mm. La vulva está situada a 0,726 mm de la extremidad anterior y en ese punto el ancho es de 0,122 mm. El ano está situado a 0,774 mm de la extremidad distal de la cola, la cual describe una pequeña curvatura regular y termina ligeramente redondeada, sin apéndices de ninguna especie; a la altura del ano la mayor anchura es de 0,113 mm.

HUÉSPED: *Hemiderma perspicillatum aztecum.*

LOCALIZACIÓN: Cavidad peritoneal.

DISTRIBUCIÓN GEOGRÁFICA: Santa Ana, Provincia de San José, C. R.

EJEMPLARES: 15 en la colección helmintológica del Laboratorio de Helmintología, Departamento de Parasitología, Facultad de Microbiología de la Universidad de Costa Rica, bajo el No. 3-4.

DISCUSION

Una vez revisada la literatura y comparadas las medidas y descripciones de los trabajos de CHANDLER (3), TRAVASSOS (5), ALMEIDA (1), OCHOTERENA y CABALLERO (4), VAZ (6) y CABALLERO (2), aunque nuestros ejemplares tengan parecido con *Litomosoides carinii* (Travassos, 1919), en vista de

una mayor profundidad del vestíbulo oral, una menor distancia entre vulva extremidàd anterior, una menor distancia entre cloaca cola, una constante relación entre espícula menor y mayor de 1:3,3 y un número constante de papilas post-cloacales (tres pares), creemos que se trate de una nueva especie.

RESUMEN

Se señala por primera vez en Costa Rica, la presencia de un representante del género *Litomosoides* Chandler, 1931, y se describe una nueva especie *Litomosoides peñai*. Los ejemplares fueron obtenidos de cavidad peritoneal de un murciélago *Hemiderma perspicillatum aztecum* (Saussure, 1860).

SUMMARY

Litomosoides peñai n. sp. is described from several specimens from the peritoneal cavity of a bat *Hemiderma perspicillatum aztecum* (Saussure, 1890).

BIBLIOGRAFIA

1. ALMEIDA, J. DE LINS
1936. Sobre un parásito de "CHEIROPTERA": *Litomosoides brasiliensis*, Rev. do Departamento Nacional da Producção Animal, 3 (1-6): 133-137.

2. CABALLERO, E.
1944. Una nueva especie del género *Litomosoides* y consideraciones acerca de los caracteres sistemáticos de las especies de este género. *An. Inst. Biol. Mex.*, 15 (2): 383-388.

3. CHANDLER, A. C.
1931. New genera and species of nematode worms. N° 2866. *Proc. U. S. Nat. Mus.* 78 (23): 23922-23931.

4. OCHOTERENA, I. y E. CABALLERO
1932. Filaria parásita de las ratas de campo, *Micropleura sigmodoni* spec. nov. *An. Inst. Biol. Mex.*, 3 (2): 123-125.

5. TRAVASSOS, L.
1919. *Filaria carinii* n. sp. *Rev. Soc. Bras. Sci.*, 3: 189.

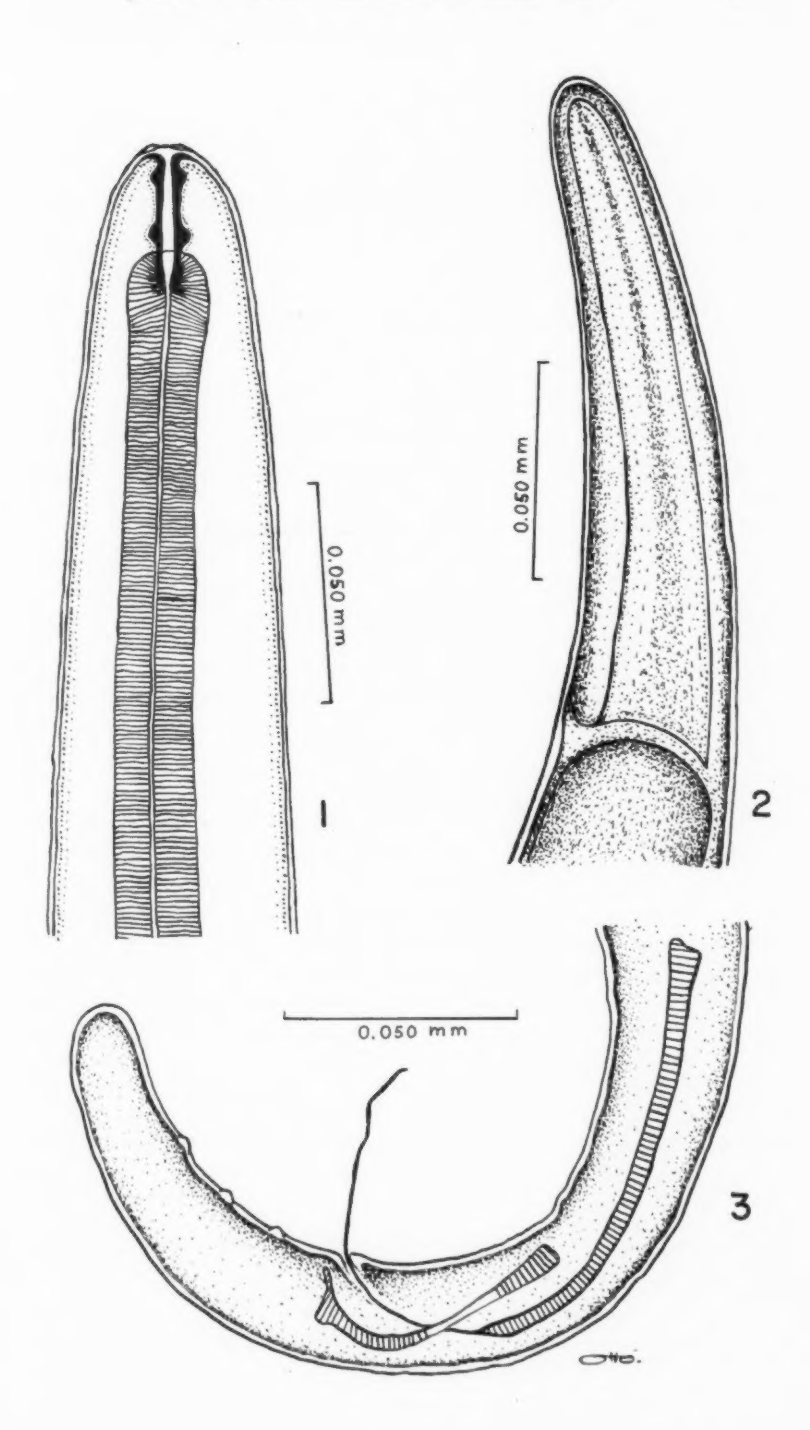
6. VAZ, Z.
1934. *Ackertia* gen. nov. for *Litomosa burgosi* de la Barrera, 1926, with notes on the synonymy and morphological variations of *Litomosoides carinii* (Travassos, 1919), *Ann. Trop. Med. Parasitol.*, 28 (2): 143-149.

7. ZELEDÓN, R. y P. L. VIETO
1957. Hallazgo de *Schizotrypanum vespertilionis* (Battaglia, 1904) en la sangre de murciélagos de Costa Rica. *Rev. Biol. Trop.*, 5 (2): 123-128.

Fig. 1: Extremidad anterior, mostrando vestíbulo oral y parte del esófago, de una preparación total (holotipo).

Fig. 2: Extremidad caudal de la hembra, de una preparación total (holotipo).

Fig. 3: Extremidad caudal del macho, mostrando tres pares de papilas post-cloacales, cloaca y espículas desiguales.


0.050 mm
1
0.050 mm
2
0.050 mm
3
Otto.



Rev. Biol. Trop., 8 (1): 69-92, 1960

Un híbrido natural de Hydrocotyle (Umbelliferae)

por

Rafael L. Rodríguez C.*

(Recibido para su publicación el 5 de abril de 1960)

Durante los dos últimos años hemos hecho varias colecciones de una *Hydrocotyle* que al principio supusimos una especie nueva. Sin embargo, la distinción entre la nueva entidad e *Hydrocotyle pusilla* tal como definen a ésta MATHIAS y CONSTANCE (14) es vaga; al acumularse suficiente material se observó que éste se encuentra siempre en localidades donde *H. mexicana* y *H. pusilla* crecen juntas o muy cercanas, y que las diferencias entre el nuevo material e *H. pusilla* tienden a acercarlo a *H. mexicana*. Estos hechos sugerían una naturaleza híbrida, corroborada luego por el estudio morfológico y estadístico, y como tal la describimos ahora. Encontrándose este material con relativa frecuencia y con caracteres bastante definidos, creemos conveniente darle un nombre.

H. mexicana Cham. & Schlecht. × *H. pusilla* A. Rich.
Hydrocotyle × *nubigena* Rodríguez, hyb. nov.

Plantae procumbentes, 0.5 - 2 metra longae, ramosae, caulibus gracilibus 0.4 - 1.5 mm crassis, hirsutis vel fere glabris. Petioli 6 -210 mm longi, distali villosi maxime parte. Foliorum laminae orbiculares cordiformes, 6 - 10 mm latae, 5 - 36 mm longae, peltatae, generatim in 7 lobulos parum profundos diffissae marginibus crenatis-serratis, quisque lobulus in partes 3 divisus, divisionibus 1 - 3 dentatis; fissura basalis profunditate variabili adest, quae rare usque ad petiolum attingit; utrinque hirsutulae secus nervos. Petioli umbilicus super faciem superiorem 1 - 4 pilis erectis ornatus. Pedunculi villosi, 10 - 70 mm longi, breviores, aequales vel raro petiolis maiores. Umbellae simplices, 6 - 17 (communiter 10) floribus. Petala alba vel diluta rosea. Ovaria viridia ac rubropunctata. Fructus immaturus subsessilis, in ambitu ellipticus; costae evidentes, acutae, paulo eminentes.

---

* Departamento de Biología, Facultad de Ciencias y Letras, Universidad de Costa Rica.

Plantas rastreras, de 0,50 a 2 m de longitud, ramificadas; tallos de 0,4 a 1,5 mm de grueso, casi glabros o con pelos ralos, adpresos o derechos. Pecíolos de 6 a 210 mm de largo, vellosos, especialmente hacia el extremo distal. Lámina de la hoja orbicular-cordiforme, de 6 a 40 × 5 a 36 mm, dividida generalmente en 7 lóbulos poco profundos, peltada, con una hendidura basal de profundidad variable que generalmente no llega hasta la unión del pecíolo con la lámina; borde crenado-serrado, cada lóbulo dividido en tres y correspondiendo a cada división 2 o 3 dientecillos. Haz y envés hirsútulos a lo largo de los nervios, la haz con pelos más escasos. Base del pecíolo marcada en la haz por 1 a 4 pelos erguidos. Pedúnculos de 10 a 70 mm, vellosos, menores, iguales, o rara vez mayores que los pecíolos. Umbela sencilla, de 6 a 17 flores, corrientemente de 10. Pétalos blancos o rosado pálido. Ovario verde punteado de rojo. Fruto elíptico en silueta, costillas evidentes.

TIPO: *R. L. Rodríguez C. 508,* potrero húmedo entre Las Nubes y Bajo de la Rosa, al E. de San Isidro de Coronado, Costa Rica, Julio 26, 1957 (CR 36.730, UC, UCR).

DISTRIBUCIÓN: Montañas centrales de Costa Rica, de 1300 a 1800 m de altitud, en laderas muy húmedas.

MATERIAL EXAMINADO: *R. L. Rodríguez C. 526-B,* entre Las Nubes y Bajo de la Rosa; *R. L. Rodríguez C. 536,* Alto de la Palma, camino de Carrillo; *E. Coen & R. L. Rodríguez C. 552,* laderas altas del Cerro de la Cruz, Alajuelita; *A. Jiménez, L. Oviedo & R. L. Rodríguez C. 613,* laderas altas del Valle de la Estrella, Cartago; *R. L. Rodríguez C. 671,* Rancho Redondo, Coronado; *S. Jiménez C. R-676,* Alto de la Palma, camino de Carrillo.

POSICION INTERMEDIA DE *HYDROCOTYLE* × *NUBIGENA*

El carácter intermedio de los ejemplares en cuestión se nota a primera vista en la morfología grosera de la hoja; pero se hace aún más evidente al comparar cuidadosamente colecciones en masa de las dos especies presuntas progenitoras y del presunto híbrido, y al estudiar la correlación de sus caracteres según los métodos de investigación desarrollados por ANDERSON (2, 3, 4, 6). En el cuadro 1 se resume un número de características morfológicas de las tres entidades, y en la figura 1 se comparan gráficamente.

TALLO. Al observarlos al fresco, se nota una diferencia marcada entre los tallos tenues y débiles de *H. pusilla* y los de *H. mexicana,* más gruesos y resistentes. En *H.* × *nubigena* los tallos tienden a ser de un grosor intermedio y a variar de consistencia desde la suavidad de *H. pusilla* hasta la resistencia de *H. mexicana,* siendo lo más frecuente encontrar tallos de 1 mm de grueso y tan resistentes como los de esta última especie. El tipo de desarrollo y ramificación es semejante en las tres entidades, si bien se diferencian claramente en el tamaño total de la planta; *H.* × *nubigena* presenta un desarrollo intermedio entre las plantitas pequeñas de *H. pusilla* y los tallos largos y bejucosos de *H. mexicana,* siendo lo más frecuente encontrar tallos de 1 mm de grueso y tan resistentes como los de esta última especie. El tipo de desarrollo y ramifica-

ción es semejante en las tres entidades, si bien se diferencian claramente en el tamaño total de la planta; *H.* × *nubigena* presenta un desarrollo intermedio entre las plantitas pequeñas de *H. pusilla* y los tallos largos y bejucosos de *H. mexicana.* En cuanto a longitud máxima, mínima y promedio de entrenudos y pecíolos, así como al ámbito de variación de unos y otros, *H. pusilla* y *H. mexicana* difieren significativamente, y *H.* × *nubigena* aparece en posición intermedia (fig. 2). En cada entidad, la relación entre el entrenudo y el pecíolo que se forma concurrentemente tiende a variar de manera semejante (fig. 3), si bien dentro de límites muy distintos para las dos especies hibridógenas, con respecto a las cuales *H.* × *nubigena* resulta nuevamente intermedia.

HOJA. Donde más claramente se ve el carácter intermedio de *H.* × *nubigena* es en las características foliares. En cuanto a tamaño promedio de la lámina y límites de variación, las tres entidades difieren entre sí de la misma manera que en cuanto a longitud de entrenudo y pecíolo (fig. 2), con idéntica posición intermedia para el presunto híbrido. Morfológicamente, las hojas orbiculares, peltadas y completamente glabras de *H. pusilla* (fig. 4) contrastan con las de *H. mexicana* reniformes, hendidas hasta el pecíolo y pelosas (fig. 6); los pelos de ésta generalmente se desarrollan sobre los nervios primarios, secundarios y aún de menor categoría, variando en número pero siempre abundantes. En la unión del pecíolo con la lámina de la hoja ("ombligo"), *H. pusilla* tiene una pequeña protuberancia glabra y *H. mexicana* un manojo de pelos generalmente abundantes y más largos que los de los nervios. En todos estos aspectos *H.* × *nubigena* muestra una notable combinación de características intermedias entre las otras dos. La variación de proporciones de sus hojas es mayor que en *H. pusilla* y menor que en *H. mexicana.* Predomina la lámina cordiforme (fig. 5) en que se combinan la posición central del pecíolo de una hoja peltada, como en *H. pusilla,* y una base hendida como la de *H. mexicana:* la hendidura es generalmente muy angosta y puede llegar hasta el pecíolo, aunque corrientemente no penetra sino hasta una distancia equivalente a desde 1/3 hasta 2/3 del radio de la hoja. La simetría es definidamente bilateral como en *H. mexicana* pero la mayor longitud relativa del lóbulo central le da un carácter enteramente diferente, que contrasta también con la forma orbicular de *H. pusilla.* El indumento es más ralo que en *H. mexicana.* Una condición intermedia más en *H.* × *nubigena* es la presencia de uno o de pocos pelos sobre el "ombligo" de la lámina, en vez de la protuberancia glabra de *H. pusilla* y del penacho abundante de *H. mexicana.* La correlación de todas estas características con las dimensiones de la hoja se muestra en las figuras 7 y 8.

INFLORESCENCIA. El pedúnculo muestra, en cuanto a longitud media y extremos de variación, las mismas diferencias entre las tres entidades que otros caracteres mensurables (fig. 2); aún más marcadas son las diferencias que se observan en cuanto al número de flores que forman la umbela. En ambos aspectos *H.* × *nubigena* ocupa la posición central del grupo. La relación entre la longitud del pecíolo y la del pedúnculo producido en el mismo nudo es muy diferente en una y otra especie hibridógena. En la figura 9 se han reunido todas

CUADRO 1

Comparación de algunas características de Hydrocotyle × nubigena con las de H. pusilla *y* H. mexicana

CARACTERISTICA		*H. pusilla*	*H. × nubigena*	*H. mexicana*
TALLO	Longitud	0,1 — 0,5 m	0,5 — 2,0 m	0,7 — 5,0 m
	Grueso	0,5 — 1,0 mm	0,5 — 2,0 mm	1,0 — 3,0 mm
	Consistencia	tenue, débil	débil a recio	recio, resistente
	Indumento	hirsuto, muy ralo	hirsuto, ralo	hirsuto, ralo
LONGITUD DE LOS ENTRENUDOS	Máxima	4,0 cm	10,4 cm	23,0 cm
	Moda	2,0 cm	5,0 cm	7,0 cm
	Media	2,35 ± 0,9 cm	5,15 ± 0,13 cm	7,82 ± 2,55 cm
	Mínima	0,5 cm	0,7 cm	2,2 cm
LONGITUD DEL PECÍOLO	Máxima	9,0 cm	20,5 cm	38,0 cm
	Moda	2,0 cm	3,0 cm	4,0 cm
	Media	2.3 ± 0,1 cm	4,90 ± 0,20 cm	6,70 ± 0,40 cm
	Mínimo	0,3 cm	0,6 cm	1,0 cm
DIÁMETRO TRANSVERSAL DE LA HOJA	Máximo	2,7 cm	4,0 cm	10,8 cm
	Moda	1,0 cm	1,6 cm	3,4 cm
	Medio	1,46 ± 0,03 cm	2.00 ± 0,04 cm	4,47 ± 0,15 cm
	Mínimo	0,3 cm	0,6 cm	1,0 cm
MORFOLOGÍA DE LA HOJA	Forma	Orbicular	Orbicular-cordiforme	Orbicular-reniforme
	Lóbulos	(6) 7 (8)	7 - 9	9 - 11
	Borde	Crenado	Crenado-dentado	Crenado-dentado
	Inserción del pecíolo	Peltada	Peltada	Lateral
	Base	Indistinta	Hendida parcialmente	Hendida
	"Ombligo"	Glabro	1 a 3 cerdas	Penacho de cerdas

	Indumento de la haz	Glabra	Ralo: 1 a 6 pelos por vena (media = 2,33).	Abundante: 10 a 60 pelos por vena (media = 20,8). Pelos en venas primarias secundarias, y menores.
	Indumento del envés	Glabro	Pelos en venas primarias y secundarias Mediano: media = 19 pelos por vena	Abundante: media = 40 pelos por vena
LONGITUD DEL PEDÚNCULO	Máxima	6,0 cm	7,0 cm	24,0 cm
	Moda	0,7 cm	2,5 cm	7,0 cm
	Media	0,84 ± 0,07 cm	2,62 ± 0,20 cm	8,24 ± 0,41 cm
	Mínima	0,2 cm	1,0 cm	2,3 cm
MORFOLOGÍA DE LA INFLORESCENCIA	Indumento del pedúnculo	Glabro (8% con pelos escasos)	Desde pelos ralos hasta hirsuto	Todos hirsutos
	Consistencia del pedúnculo	Tenue	Medianamente débil	Tan recio como los pecíolos.
	Razón (media) pedúnculo/pecíolo de un mismo nudo	0,365	0,534	1.222
	Pedicelos	Nulos a 1 mm	1 mm	2 a 7 mm
NÚMERO DE FLORES POR UMBELA	Máximo	8	18	66
	Moda	3	10	37
	Media	3,0 ± 0,09	11,32 ± 0,45	41,2 ± 0,8
	Mínimo	1	6	13
FLOR	Pétalos	Carmín a rosado	Rosado, punteado de rosa, rosado en el ápice, o blanco transparente	Blanco transparente, verdoso o amarillento
	Ovario	Verde punteado de rojo	Verde, corrientemente punteado de rojo	Verde
FRUTO	Color durante maduración	Pardo anaranjado intenso	Verde; las manchas rojas desaparecen. No se presenta el color anaranjado	Verde
	Color maduro	Terroso claro	Terroso	Terroso
	Costillas	Obsoletas	Marcadas	Bien marcadas

las medidas efectuadas en el material disponible, usando como coordenadas una y otra medida, y en ella resalta una vez más la condición intermedia de *H.* × *nubigena*. Esta posición intermedia se muestra aún más claramente en la correlación entre longitud del pedúnculo y numero de flores por umbela (fig. 10).

FLOR Y FRUTO. La corola de color rosado pálido o blanco nos hace considerar a *H.* × *nubigena* más próxima a *H. pusilla* que a *H. mexicana,* aunque siempre en posición intermedia. El ovario de *H. pusilla* es abultado, ovoide, y en material fresco son muy llamativas las manchas de intenso color rojo sobre el fondo verde; ROSE y STANDLEY (15) lo describieron como "obscurely tuberculate". Al madurar toma un color pardo anaranjado fuerte que finalmente se vuelve terroso pálido. *H.* × *nubigena* tiene corrientemente las mismas manchas rojas, pero en el proceso de maduración no se presenta el color anaranjado; las manchas rojas desaparecen y el color verde oscuro pasa a terroso como en *H. mexicana.*

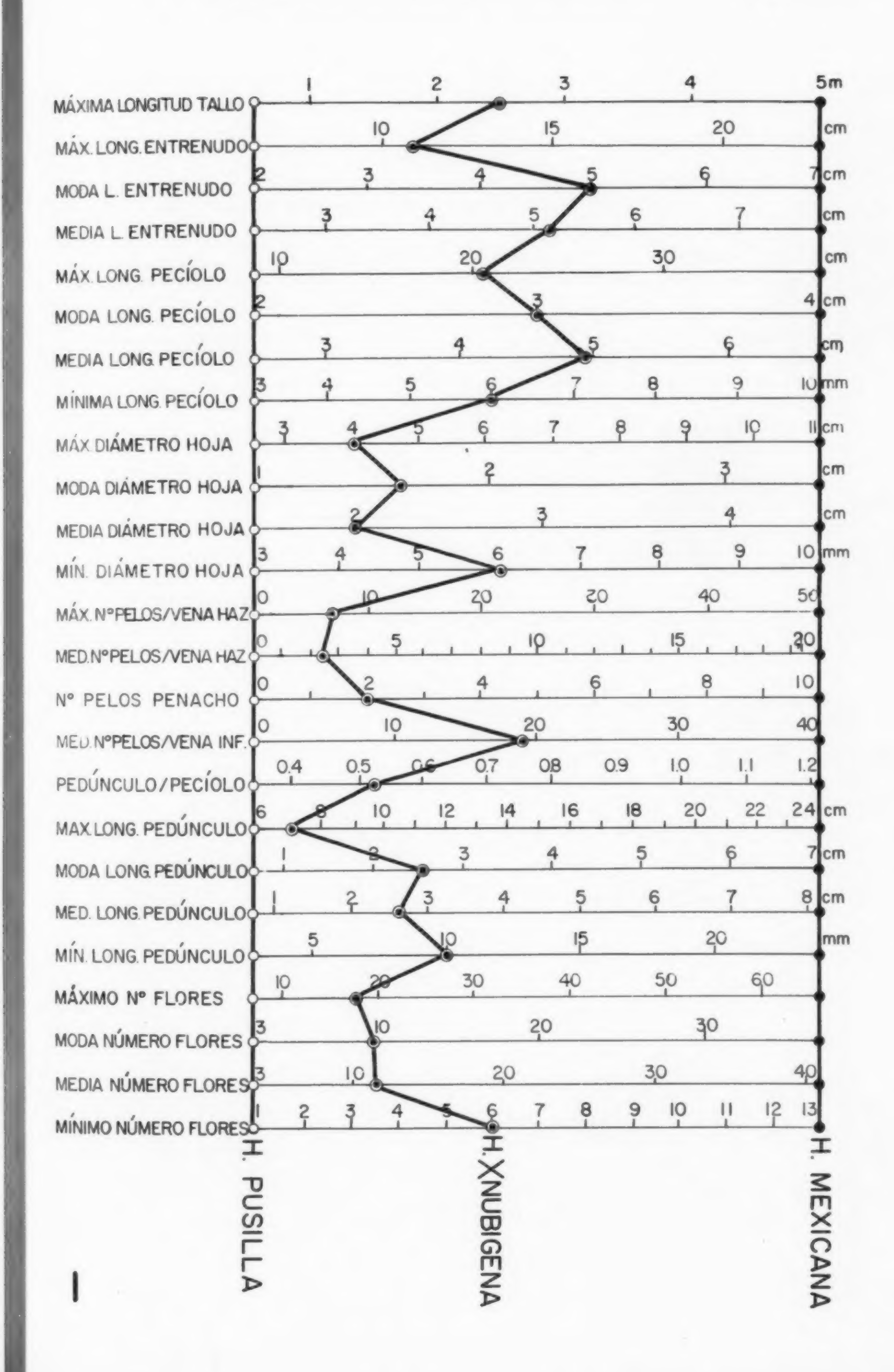
INDICE DE HIBRIDACION

Para investigar la ocurrencia de introgresión entre estas entidades (cf. ANDERSON, 2, 3, 4, 6) se hizo una lista de 11 caracteres en que *H. mexicana* e *H. pusilla* se diferencian claramente: longitud y consistencia del tallo, longitud del pecíolo, diámetro de la hoja, hoja orbicular peltada vs. reniforme hendida, presencia o ausencia de un penacho de tricomas sobre el "ombligo" de la lámina, indumento en la haz de la hoja, indumento en el envés, relación entre la longitud del pedúnculo y del pecíolo, longitud del pedúnculo, número de flores por umbela y color de las flores. Para cada uno de estos caracteres se asignó un valor de 0 a la condición típica de *H. pusilla* y de 6 a la de *H. mexicana.* Se valoró individualmente cada planta de una y de otra especie, así como de *H.* × *nubigena,* de acuerdo con la escala establecida, dando valores intermedios según el caso. En la comparación se usaron 33 plantas de *H. mexicana,* de 12 localidades; 37 de *H.* × *nubigena,* de 5 localidades; y 51 de *H. pusilla,* de 17 localidades. La figura 11 muestra gráficamente los resultados, expresados como porcentaje de la muestra de cada entidad.

En cuatro de las cinco localidades parece haber introgresión de genes de *H. mexicana* en la población de *H. pusilla.* Los ejemplares de ésta con índices superiores a 1 provienen todos de las localidades donde se encontró el híbrido; los valores obtenidos se deben a combinaciones diferentes, desde plantas típicas de *H. pusilla* con tallos anormalmente gruesos y pecíolos anormalmente grandes hasta *H.* × *nubigena* típica con hojas y pecíolos muy pequeños. Las colecciones número 526, de Las Nubes, formaban aparentemente parte de

---

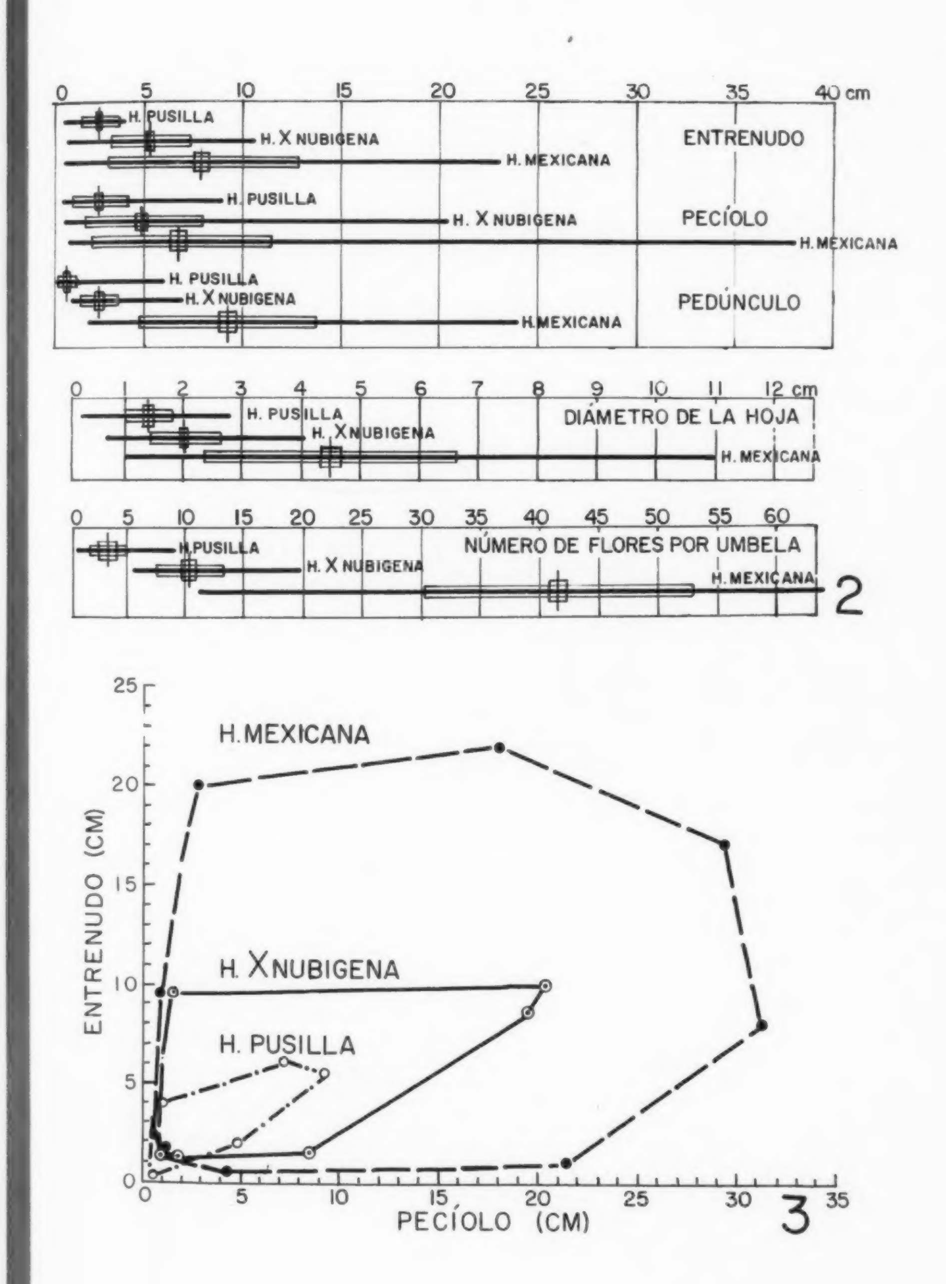
Fig. 1: Posición intermedia de *H.* × *nubigena* con relación a sus presuntos progenitores. Se han representado con dos líneas paralelas las condiciones características de *H. mexicana* y *H. pusilla.* En cada línea transversal se ha indicado una característica mensurable, haciéndose variar la escala para que las cifras correspondientes a una y otra especie coinciden con las líneas horizontales. Sobre esa escala se ha indicado el valor correspondiente al híbrido.


MÁXIMA LONGITUD TALLO
MÁX. LONG. ENTRENUDO
MODA L. ENTRENUDO
MEDIA L. ENTRENUDO
MÁX. LONG. PECÍOLO
MODA LONG. PECÍOLO
MEDIA LONG. PECÍOLO
MÍNIMA LONG. PECÍOLO
MÁX. DIÁMETRO HOJA
MODA DIÁMETRO HOJA
MEDIA DIÁMETRO HOJA
MÍN. DIÁMETRO HOJA
MÁX. Nº PELOS/VENA HAZ
MED. Nº PELOS/VENA HAZ
Nº PELOS PENACHO
MED. Nº PELOS/VENA INF.
PEDÚNCULO/PECÍOLO
MAX. LONG. PEDÚNCULO
MODA LONG. PEDÚNCULO
MED. LONG. PEDÚNCULO
MÍN. LONG. PEDÚNCULO
MÁXIMO Nº FLORES
MODA NÚMERO FLORES
MEDIA NÚMERO FLORES
MÍNIMO NÚMERO FLORES
H. PUSILLA
H. X NUBIGENA
H. MEXICANA
5m
cm
mm

I

Fig. 2: Análisis estadístico gráfico de algunas características mensurables de *Hydrocotyle*, en el cual se nota la posición intermedia de *H.* × *nubigena*.

Fig. 3: Comparación entre la longitud de los entrenudos y la de los pecíolos producidos con ellos por plantas de *H. mexicana*, *H. pusilla* e *H.* × *nubigena*. La relación entre ambas dimensiones varía de manera similar en las tres entidades, pero los ámbitos de variación son bien diferentes; nótese la posición intermedia del híbrido.


0 5 10 15 20 25 30 35 40 cm
H. PUSILLA
H. X NUBIGENA
H. MEXICANA
ENTRENUDO
H. PUSILLA
H. X NUBIGENA
H. MEXICANA
PECÍOLO
H. PUSILLA
H. X NUBIGENA
H. MEXICANA
PEDÚNCULO
0 1 2 3 4 5 6 7 8 9 10 11 12 cm
H. PUSILLA
H. X NUBIGENA
H. MEXICANA
DIÁMETRO DE LA HOJA
0 5 10 15 20 25 30 35 40 45 50 55 60
H. PUSILLA
H. X NUBIGENA
H. MEXICANA
NÚMERO DE FLORES POR UMBELA
2
H. MEXICANA
H. X NUBIGENA
H. PUSILLA
ENTRENUDO (CM)
PECÍOLO (CM)
0 5 10 15 20 25
0 5 10 15 20 25 30 35
3

Figs. 4 - 6: **Variaciones observadas** en el tamaño, forma y pubescencia de las hojas de *Hydrocotyle*.

Fig. 4: *Hydrocotyle pusilla*. Nótese el tamaño pequeño, la hoja orbicular peltada y la superficie glabra.

Fig. 5: *Hydrocotyle* × *nubigena*. Nótese variación del tamaño, la hoja peltada cordiforme con hendidura basal muy variable, pubescencia escasa y número escaso de pelos sobre el pecíolo.

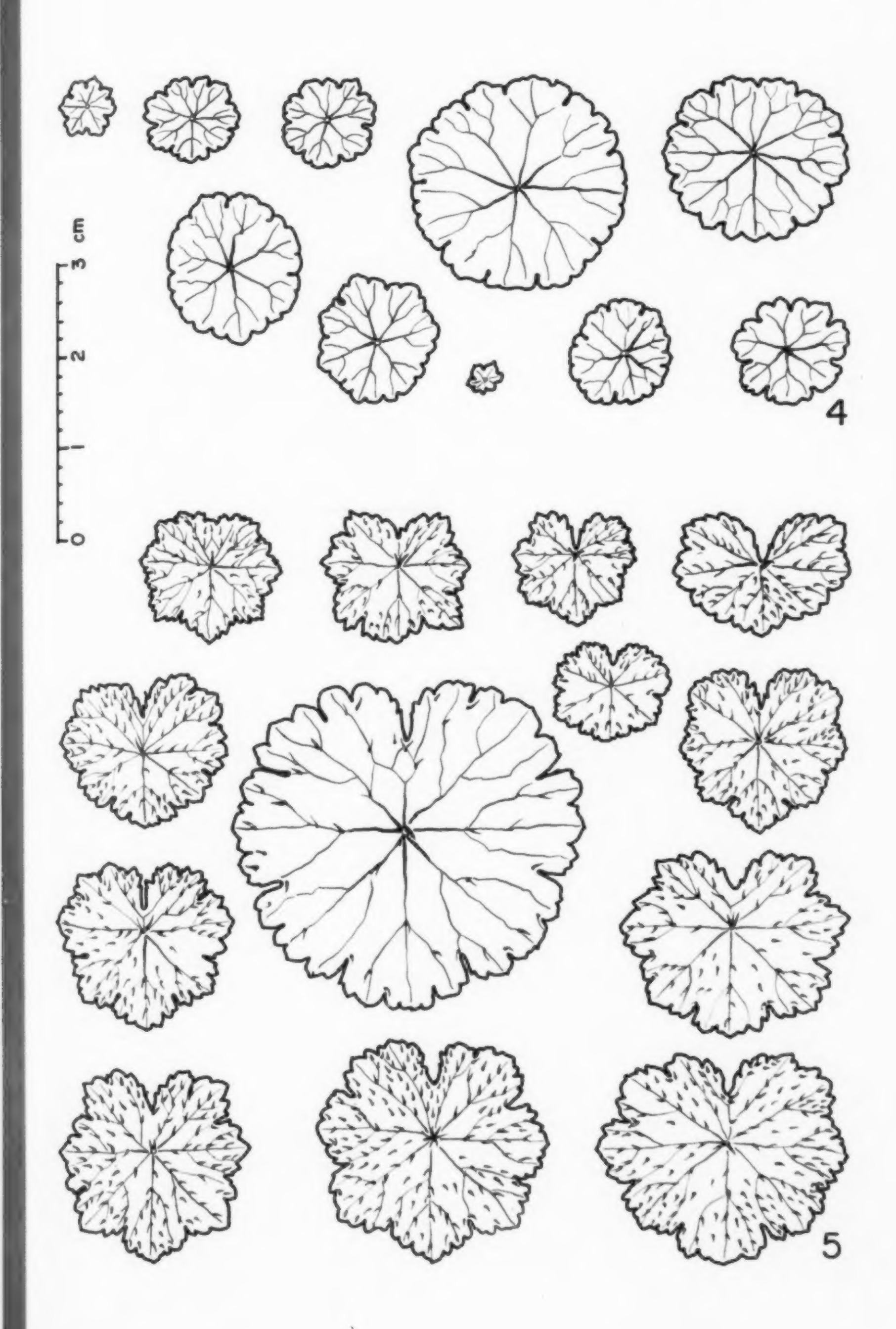
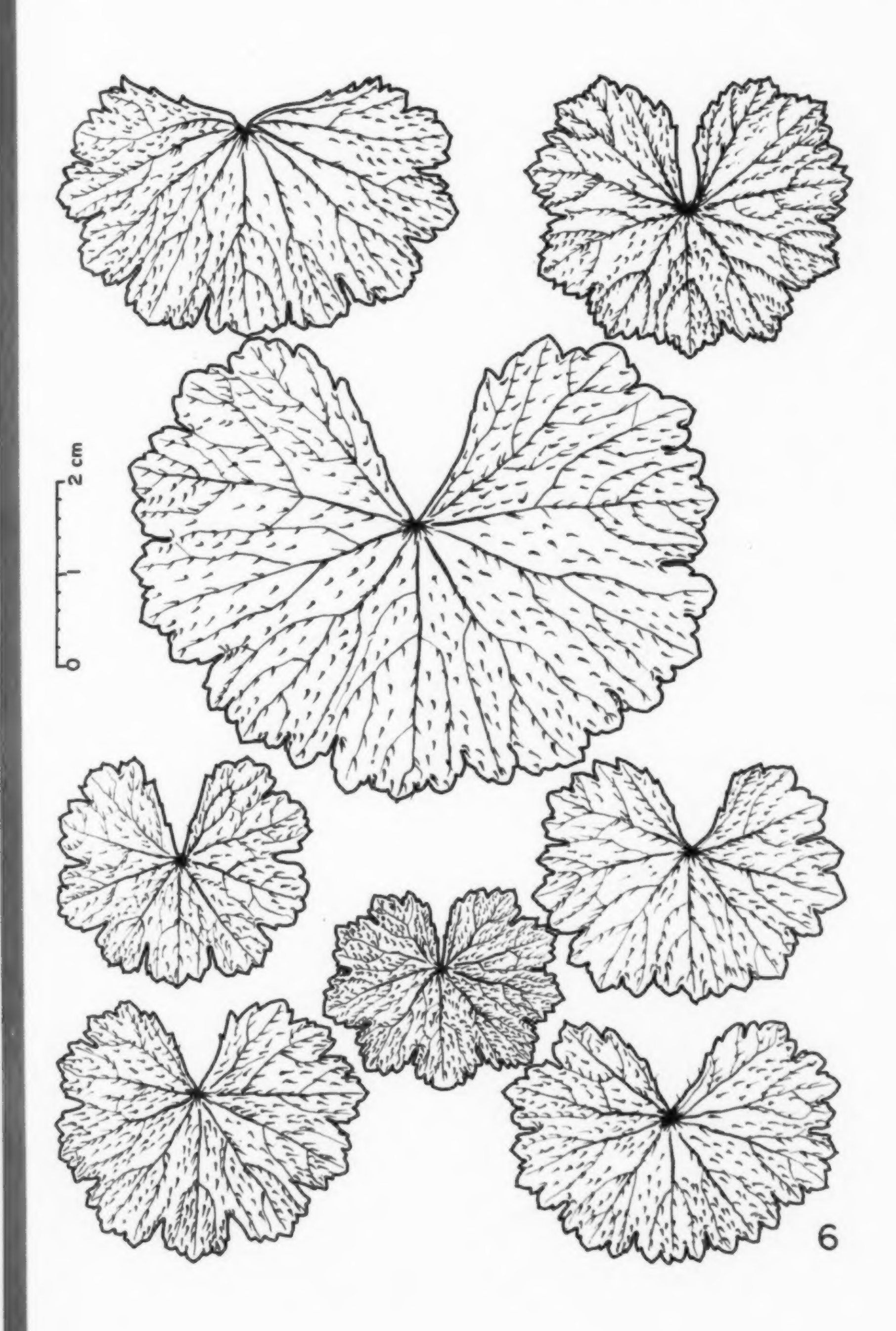

3 cm
2
1
0
4
5

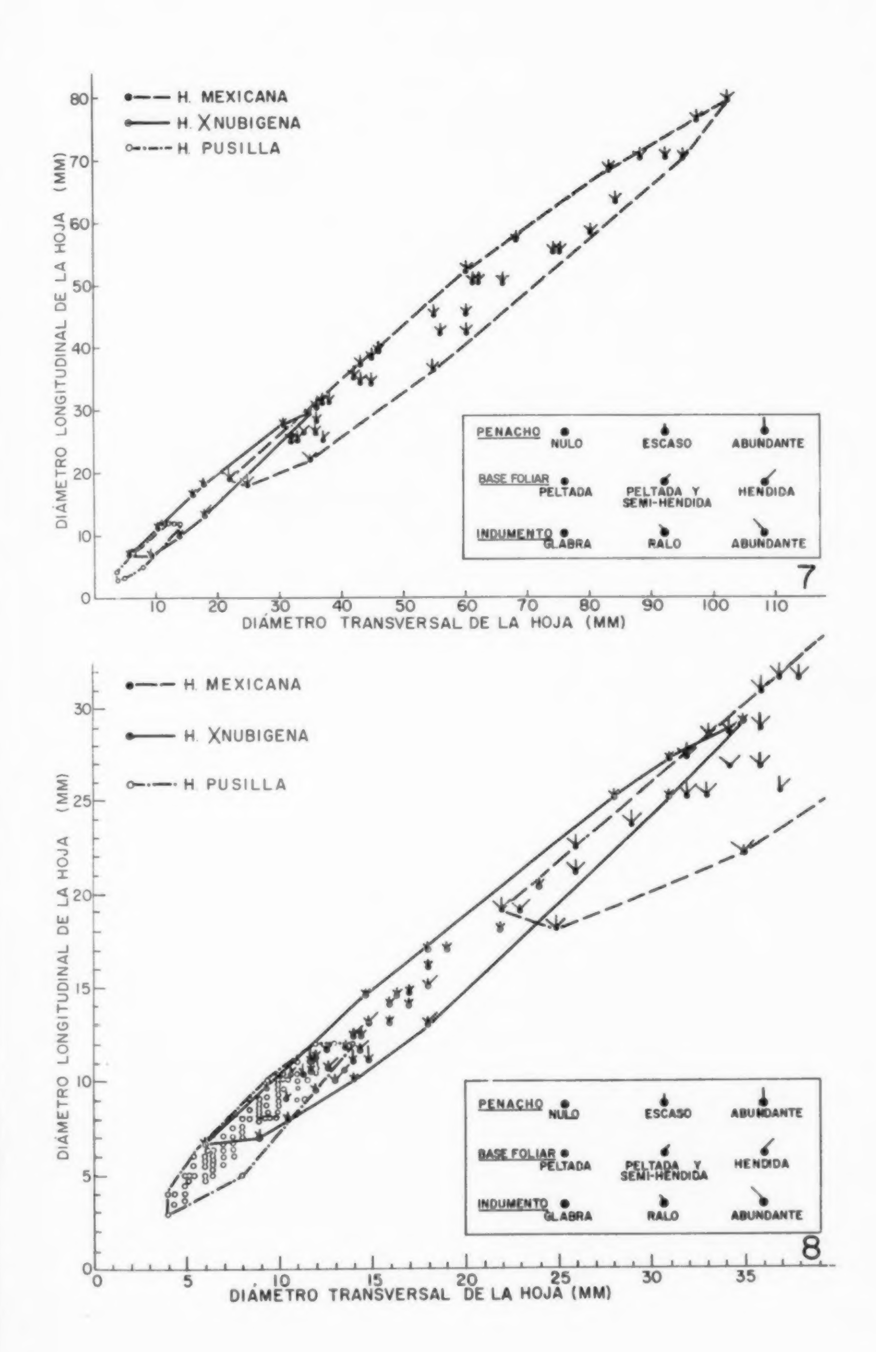
Fig. 6: *Hydrocotyle mexicana*. Nótese el mayor tamaño de las hojas, la lámina hendida hasta el pecíolo, la pubescencia abundante y el mechón de pelos sobre el pecíolo.


2 cm
0
6

Figs. 7 - 8: Comparación gráfica de las características foliares de colecciones en masa de *Hydrocotyle* de una misma localidad.

Fig. 7: Comparación de la totalidad de hojas de los ejemplares recogidos en el Alto de La Palma.

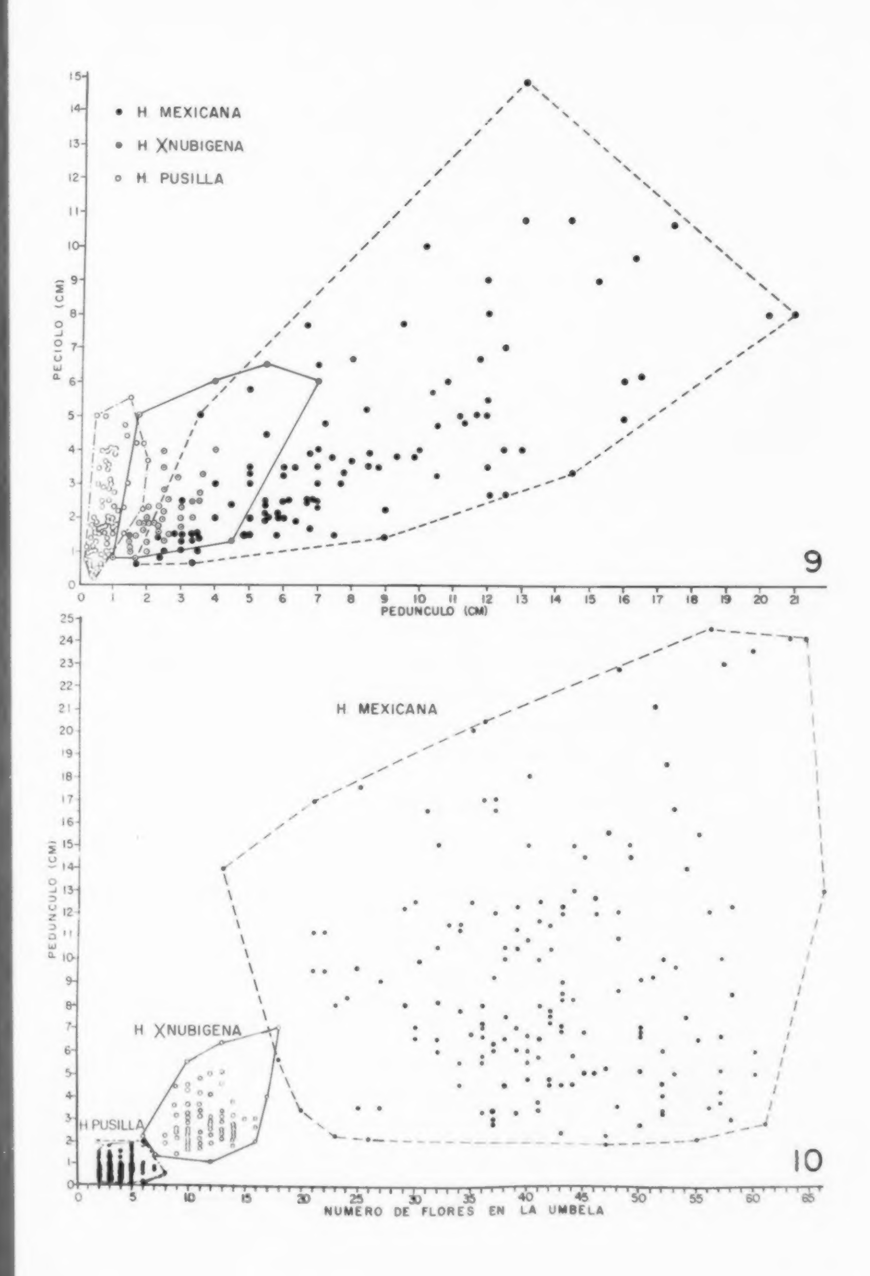
Fig. 8: Detalle de la fig. 7, de la que se ha eliminado parte de los datos correspondientes a *H. mexicana* para permitir mayor claridad en la indicación de las variaciones de *H.* × *nubigena*.


H. MEXICANA
H. X NUBIGENA
H. PUSILLA
DIÁMETRO LONGITUDINAL DE LA HOJA (MM)
DIÁMETRO TRANSVERSAL DE LA HOJA (MM)
PENACHO
NULO
ESCASO
ABUNDANTE
BASE FOLIAR
PELTADA
PELTADA Y SEMI-HENDIDA
HENDIDA
INDUMENTO
GLABRA
RALO
ABUNDANTE
7
H. MEXICANA
H. X NUBIGENA
H. PUSILLA
DIÁMETRO LONGITUDINAL DE LA HOJA (MM)
DIÁMETRO TRANSVERSAL DE LA HOJA (MM)
PENACHO
NULO
ESCASO
ABUNDANTE
BASE FOLIAR
PELTADA
PELTADA Y SEMI-HENDIDA
HENDIDA
INDUMENTO
GLABRA
RALO
ABUNDANTE
8

Figs. 9 - 10: **Características** de la inflorescencia de *Hydrocotyle*.

Fig. 9: **Relación** entre la longitud del pecíolo y la del pedúnculo que salen del mismo nudo.

Fig. 10: **Relación** entre la longitud del pedúnculo y el número de flores en la umbela.



H. MEXICANA
H. XNUBIGENA
H. PUSILLA
PECIOLO (CM)
PEDUNCULO (CM)
9
H. MEXICANA
H. XNUBIGENA
H PUSILLA
PEDUNCULO (CM)
NUMERO DE FLORES EN LA UMBELA
10

%
70

60

50

40

30

20

10

0

Fi

un
pr
de
co
U
de
co

co
(
ot
S
er
d
n
ca
es
co
co
e
n
c
l

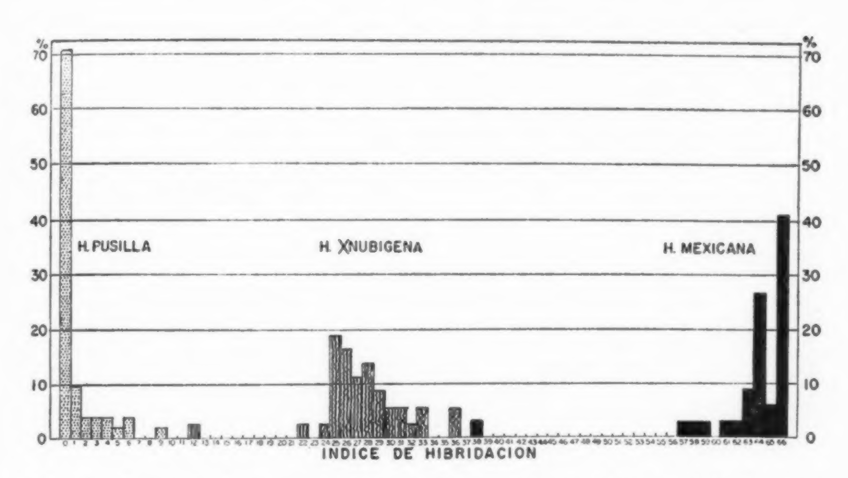


Fig. 11: Frecuencia de valores del índice de hibridación en *Hydrocotyle*, indicada como porcentaje de la muestra observada.

una colonia parcialmente segregante, pues contenían ejemplares típicos de *H. pusilla* con tallo recio (526-A, valores de 5 y 6), intermedios (526-B, valor de 22), de *H.* × *nubigena* típica (526-B, valores de 26 y 27) y ejemplares con la mayoría de las hojas hendidas hasta el pecíolo (526-C, valor de 29). Unicamente en una localidad, Rancho Redondo, se encontró indicios abundantes de introgresión de *H. pusilla* en *H. mexicana.* De allí son los ejemplares de ésta con menor índice, y los de mayor índice del híbrido.

DISCUSION

La importancia de la hibridación en la evolución de las plantas ha sido comentada ampliamente por ANDERSON (2, 4, 5, 6), ANDERSON y STEBBINS (7), CONSTANCE (9, 10), GRANT (11, 12) y STEBBINS (17, 18, 19), entre otros. En las umbelíferas, ya BELL (8) ha apuntado el origen híbrido de una *Sanicula* norteamericana; este es el primer informe, según creo, de hibridación en *Hydrocotyle.* Los datos aportados atestiguan claramente la posición intermedia de *H.* × *nubigena* respecto a las dos especies que se consideran sus progenitores. En todos los casos, las comparaciones se han hecho con el material de cada entidad tal como apareció en nuestras colecciones. Es necesario advertir esto particularmente en relación con *H. pusilla.* Las descripciones de esta especie que dan STANDLEY (16) o MATHIAS y CONSTANCE (14) harían parecer el contraste con *H.* × *nubigena* menos marcado de lo que en realidad es, por ejemplo, al definir la hoja como "glabra a ralamente vellosa" en la haz. El material costarricense que hemos visto ha sido constantemente glabro, con excepción de dos hojas juveniles que tenían 3 y 4 pelos por nervio primario en la haz. En general, los ejemplares de *H. pusilla* recogidos en 18 localidades

diferentes de la región central de Costa Rica corresponden exactamente a la descripción que ROSE y STANDLEY (15) dieron de *H. costarricensis,* posteriormente reducida a sinónimo de *H. pusilla.* El contraste de *H.* × *nubigena* con este material sí es bien marcado en todos los aspectos apuntados.

En todas las especies del género *Hydrocotyle* se observa gran variación en la longitud de los entrenudos y pecíolos, así como en el desarrollo de las láminas de la hoja, la longitud del pedúnculo, etc. Por esta razón, al comparar las entidades en cuestión se han medido todos los elementos desarrollados comprendidos en las colecciones en masa, evitando escoger un número de elementos "representativos" o "típicos" en cuya selección pudiese entrar un factor subjetivo. Las figuras 7 y 8 corresponden al material de una sola localidad, el Alto de La Palma; se hicieron diagramas separados de las colecciones de cada localidad, para evitar el posible efecto de condiciones ambientales diferentes, que parecen repercutir más fuertemente sobre el desarrollo del pecíolo y de la lámina que sobre otros caracteres. Los diagramas correspondientes a las demás localidades son esencialmente similares a los que publicamos.

La enorme variabilidad de tamaño de la hoja de *H. mexicana* se observa en la figura 7; se hizo necesario reducir mucho la escala del diagrama para poder incluir en él los extremos de esa variación. Como, aparte del tamaño, las características morfológicas de *H. mexicana* son muy constantes, especialmente en las hojas grandes, en la figura 8 se ha eliminado parte del área cubierta por el material de dicha especie para permitir una escala mayor en que se pueda ver más claramente la variación y las combinaciones de caracteres de la muestra de *H.* × *nubigena.*

Los indicios de introgresión han sido relativamente pocos; en cuatro localidades se nota más introgresión hacia *H. pusilla* que hacia *H. mexicana,* mientras que en una localidad se da el caso contrario. Este último permite descartar la suposición de una barrera de esterilidad entre *H. mexicana* y la nueva entidad, como la de ciertos casos citados por STEBBINS (17) o según un mecanismo como el descrito por GRANT (12); más bien, la explicación más aceptable parece ser que la introgresión se da en cada localidad desde el híbrido a la especie cuyos ejemplares están más cercanos, probablemente por ser de esa especie las plantas que produjeron la semilla híbrida al recibir el polen de la otra. Coincidiendo con fenómenos comentados por STEBBINS (op. cit.), no se encuentran indicios de libre segregación de los caracteres comparados, sino una repetición, en localidades distintas entre sí, de un modelo bastante estable, excepto en las colecciones 526-A, B y C ya descritas. Cabe suponer a este respecto la producción en cada caso de híbridos de F_1 viables, con segregación parcial en la F_2; o bien, que la mayoría de las colecciones de *H.* × *nubigena* correspondan a un genotipo viable estabilizado después de la F_1, por medio del cual se va realizando muy lentamente la introgresión hacia las poblaciones más cercanas de las especies progenitoras.

Es muy interesante la distribución del híbrido en relación con la de las otras dos especies. *Hydrocotyle pusilla* se encuentra en Costa Rica en la Meseta Central y montañas vecinas, en paredones húmedos, orillas de ríos y quebradas,

potreros y orillas de camino húmedos; generalmente en lugares despejados, con buena iluminación y bastante humedad. *Hydrocotyle mexicana* es preferentemente una especie del suelo de los bosques, generalmente desde los 1300 m de altitud. Se le ve invadir los potreros y seguir por los paredones y caminos, a la sombra de arboledas y matorrales o entre la hierba en lugares muy húmedos y frescos. Todas las muestras de *H.* × *nubigena* encontradas hasta la hora lo fueron en localidades en que *H. pusilla* e *H. mexicana* crecían juntos en potreros con arboledas o bosquecillos adyacentes, desde los cuales la segunda especie había llegado al terreno despejado, o bien a la orilla de caminos (Cascajal, La Palma) que habían favorecido la aproximación. En cierta manera, es este un caso típico del híbrido interespecífico que sigue a la alteración de las condiciones naturales por el hombre—a la "hibridación del ambiente" que dijera ANDERSON (1, 2, 5). Pero hay lugares que reúnen todas esas condiciones—presencia de las dos especies hibridógenas, y aproximación de sus ambientes típicos gracias a la intervención humana—sin que se encuentre rastro del híbrido. Tal es el caso de la región al N y NE de San Ramón de Tres Ríos, donde cuatro excursiones en distintas épocas del año han sido infructuosas aunque *H. mexicana* y *H. pusilla* se encuentran a 10 m de distancia en lados opuestos del camino o en distintos puntos del mismo paredón. Corresponde esta región al límite de la zona de *bosque húmedo montano bajo* mencionada por HOLDRIDGE (13), formación de poca extensión desarrollada al abrigo del macizo Irazú-Turrialba, mientras que las localidades donde sí se ha encontrado el híbrido están comprendidas en la formación de *bosque muy húmedo montano bajo* (La Palma, La Estrella, Rancho Redondo, Cerro de la Cruz) o en la de *bosque pluvial montano bajo* (Cascajal, Las Nubes). Todas estas localidades están en pendientes moderadas o agudas, orientadas principalmente hacia el NE y que la mayor parte del tiempo están envueltas por neblina o bañadas por lloviznas y lluvias por recibir de frente los vientos reinantes. Cabe preguntarse, pues, si la restricción del híbrido a estas regiones se debe a sus límites de tolerancia particulares, resultantes de las combinaciones nuevas de genes, o a otras causas. Arguye en contra de la primera suposición el éxito obtenido al transplantar un fragmento de la colección 613, de los montes de La Estrella, a Betania de Montes de Oca, donde ha seguido creciendo por más de un año con pleno vigor y manteniendo las mismas características morfológicas que tenía en su lugar de origen. Aunque se le da suficiente agua, es indudable que las temperaturas media, máxima y mínima en Montes de Oca son superiores a las de los montes de La Estrella, y que la humedad del aire es menor. Este transplante también contradice la suposición de que *H.* × *nubigena* sea una forma inducida en *H. pusilla*, o en *H. mexicana*, por condiciones ambientales. Es más probable, pues, que la restricción observada del híbrido tenga una de las siguientes explicaciones:

a) Normalmente, cada una de las especies hibridógenas está mejor adaptada a su ambiente particular que el híbrido (cf. ANDERSON, 1, 2, 5,

STEBBINS, 17, 19) y solamente en un ambiente despejado y bañado por las lloviznas logra éste competir con aquéllas.

b) Normalmente, *H. mexicana* crece a la sombra y *H. pusilla* entre la hierba asoleada, como en San Ramón de Tres Ríos, y solamente en el ambiente de gran humedad y lloviznas abundantes logran crecer suficientemente cerca para que la hibridación sea posible.

c) Tal vez sea necesario el ambiente de gran humedad y lloviznas para que los mismos insectos polinizadores visiten a ejemplares cercanos de ambas especies hibridógenas.

Hasta la fecha no tenemos recuentos de cromosomas de ninguna especie costarricense de *Hydrocotyle*, por lo que está pendiente la confirmación citológica de la naturaleza híbrida de *H.* × *nubigena*. De observaciones preliminares comunicadas por el Dr. C. R. Bell se desprende que se trata de poliploides naturales en casi todos los casos; la estabilización de un genotipo bien adaptado a las condiciones del ambiente es concebible como resultado del cruzamiento de dos poliploides naturales. Quedan pendientes, también, pruebas de progenie que vendrían a aclarar mucho la naturaleza de este taxon. Esperamos obtener tanto observaciones citológicas como pruebas de progenie del ejemplar transplantado, al tiempo que se continuará la investigación en el campo.

AGRADECIMIENTO

El autor consigna gustoso su agradecimiento a sus colegas y compañeros de excursión, Dr. John de Abate, Prof. Leonel Oviedo e Ing. Elliott Coen, de la Universidad de Costa Rica, Sr. Salvador Jiménez C., e Ing. Agr. Alfonso Jiménez, del Museo Nacional, por su colaboración en la recolección de ejemplares; en la Universidad de California, a la Dra. Mildred Mathias (Los Angeles), Srta. Annetta Carter y Dr. Lincoln Constance (Berkeley) por su fina atención a varias cosultas, y a la Srta. Francia H. Chisaki (Berkeley) por su valiosa cooperación en consultas y en la comparación del material de estudio con el del herbario de aquella institución. Igualmente al Prof. Arturo Agüero la revisión de la descripción en latín.

RESUMEN

Se describe *Hydrocotyle* × *nubigena* Rodríguez, hyb. nov., como híbrido natural de *H. mexicana* Cham. & Schlecht. × *H. pusilla* A. Rich., recogido en cinco localidades de la región central de Costa Rica donde ambas especies progenitoras se reúnen en un ambiente con neblina y lloviznas abundantes, y cuyos caracteres son intermedios entre dichas especies.

SUMMARY

Hydrocotyle × *nubigena* Rodríguez, hyb. nov. is described as a natural

hybrid of *H. mexicana* Cham. & Schlecht. × *H. pusilla* A. Rich, collected in five sites of the central highlands of Costa Rica where the altitude and the NE orientation of the slope cause abundant fog, drizzles and rain throughout most of the year, and where the parental species, one occurring usually in forest floor, in the shade of roadside tangles or second growth vegetation, or on very humid pastures, the other in open pastures, stream banks or along trails, are brought together by the climate, human disturbance of the environment, or both. The new taxon is intermediate between the presumed parent species in size, stem thickness and texture, internode length, petiole length, shape of the lamina, peltate character of one and basal sinus of the other, vesture of both leaf surfaces, tuft over base of lamina, size of lamina, peduncle length, peduncle/petiole ratio, number of flowers in umbel, petal color, ovary color, and fruit.

BIBLIOGRAFIA

1. ANDERSON, E.
1948. Hybridization of the habitat. *Evolution*, 2: 1-9.

2. ANDERSON, E.
1949. *Introgressive hybridization*. ix + 109 pp. J. Wiley & Sons, N. Y.

3. ANDERSON, E.
1956. Natural history, statistics, and applied mathematics. *Am. Jour. Bot.*, 43 (10): 882-889.

4. ANDERSON, E.
1956. Character association analysis as a tool for the plant breeder. En *Genetics in plant breeding. Brookhaven symposia in biology*. 9: 123-140.

5. ANDERSON, E.
1957. Man as a maker of new plants and new plant communities. *Smithsonian Institution Report for 1956* (Publ. 4286): 461-479.

6. ANDERSON, E.
1957. A semigraphical method for the analysis of complex problems. *Proc. Nat. Acad. Sci. (Wash.)* 43 (10): 923-927.

7. ANDERSON, E., & G. L. STEBBINS
1954. Evolution as an evolutionary stimulus. *Evolution*, 8 (4): 378-388.

8. BELL, C. R.
1954. The *Sanicula crassicaulis* complex (Umbelliferae): a study of variation and polyploidy. *Univ. Calif. Publ. Bot.*, 27 (3): 133-230.

9. CONSTANCE, L.
1950. Some specific relationships in *Phacelia*, subgenus *Cosmanthus*. *Proc. Am. Acad. Sci.*, 78 (3): 135-147.

10. CONSTANCE, L.
1953. The role of plant ecology in biosystematics. *Ecology*, 34 (3): 642-649.

11. GRANT, V.
1957. The plant species in theory and practice. En *The species problem*. A. A. A.

S., Washington, D. C.: 39-80.

12. GRANT, V.
1958. The regulation of recombination in plants. *Cold Springs Harbor Symp. Quant. Biol.*, 23: 337-363.

13. HOLDRIDGE, L.
1953. La vegetación de Costa Rica. En *Atlas estadístico de Costa Rica*. Dir. Gral. Estadística y Censos, M.A.I., San José, C. R.: 32-33.

14. MATHIAS, MILDRED, & L. CONSTANCE
1944. Umbelliferae. *North American Flora*. 28B: 43-295.

15. ROSE, J. N., & P. C. STANDLEY
1927. The Central American species of Hydrocotyle. *Jour. Wash. Acad.*, 17 (8): 194-197.

16. STANDLEY, P. C.
1937-1938. *Flora of Costa Rica*. Field Mus. Nat. Hist., Bot. ser., 18, 4 vols., 1616 pp.

17. STEBBINS, G. L.
1950. *Variation and evolution in plants*. xx + 643 pp. Columbia Univ. Press, N. Y.

18. STEBBINS, G. L.
1957. The hybrid origin of microspecies in the *Elymus glaucus* complex. *Cytologia* (Suppl. vol.), *Proc. Int. Genetics Symp., 1956*: 336-340.

19. STEBBINS, G. L.
1959. The role of hybridization in evolution. *Proc. Am. Philos. Soc.*, 103 (2): 231-251.

Rev. Biol. Trop., 8 (1): 93-123, 1960

The phylogeny and relationships between the insect orders

by

Alvaro Wille*

(Received for publication May 9, 1960)

The phylogeny and the interrelationships of the insect orders always remain a matter of great interest to the general entomologist. The number of papers that have been published on this subject is very large. Very unfortunately, however, most of these papers are too brief, containing only a few facts to support the views which the author has adopted, or they deal only with one order or a group of more or less interrelated orders. The purpose of this paper is to offer a general account of the origin of the insects and the known relationships between the insect orders, giving as many facts as possible to show these interrelationships.

As might be expected, current interpretations of the phylogeny of, and connections between the insect orders are not definitive, and many of our present views may have to be changed in the future, as knowledge progresses. Although this type of studies always reveals many unsolved problems, the large amount of data accumulated in the last few decades allows us to speculate on these matters, and in doing so, we may put some order and understanding into these chaotic accumulations of facts. This may explain, perhaps, the large number of papers on this subject, and will always justify further additions.

Since this is not a detailed revision of all the known views available in the literature, I have adopted those which the facts best seem to support. However, in those cases in which the data can be equally interpreted in different ways, the various interpretations are discussed.

ACKNOWLEDGMENTS

The present work was started as a seminar, given a few years ago at the University of Kansas and the final form was completed at the University of Costa Rica, Facultad de Agronomía.

* Departamento de Entomología, Facultad de Agronomía, Universidad de Costa Rica.

I wish to thank Dr. C. D. Michener, Dr. G. Byers, and Dr. R. Matsuda, of the University of Kansas, for reading the manuscript and offering some suggestions.

Thanks are due also to Dr. H. V. Daly, of Louisiana State University, for checking many of the bibliographical citations and to Dr. R. L. Rodríguez, of the University of Costa Rica, for his special interest in helping to draw the phylogenetic tree and proofreading the manuscript.

Finally, I wish to thank my wife, Mrs. María Eugenia B. de Wille, for typing and proofreading much of this paper.

THE ORIGIN OF THE INSECTS*

The insects arose about 350 million years ago, probably in the lower Devonian or earlier. Our knowledge of the morphology of arthropods seems to indicate that the closest relatives of insects are the myriapods, of which the Symphyla have the greater number of characters in common with the primitive insects. This does not mean that the insects arose directly from the Symphyla or other myriapod classes, but rather that the insects and myriapods were derived from a common stock or from myriapod-like arthropods. It is also possible that insects originated by neoteny from myriapod-like arthropods, at least there are some evidences for this. Evolution by neoteny is probably much more common in the animal kingdom than most biologists have supposed. There are two main types of neoteny, one in which the rate of development of the body remains more of less constant but the reproductive system is accelerated, and the other in which the rate of development of the body is retarded but there is no acceleration of the rate of development of the reproductive organs. The first type of neoteny leads to specialization, usually resulting in a simplification often associated with parasitism, and is of little importance for phylogeny. This happens, as DE BEER (1) has pointed out, because "if the reproductive system is accelerated, the structure of the body will be less fully developed when the animal is sexually mature than was the body of the ancestor. On the other hand, if there is no acceleration of the rate of development of the reproductive system, but only a slower rate of succession of ontogenetic stages of the body, the latter will be no less 'well made' for retaining the larval plan." We know that evolution by neoteny probably took place in a number of cases. Of these we can mention the chordates from the echinoderms, and the appendicularians from the ascidians. It is possible that even man evolved neotenically from former anthropoid-like primates (for details see DE BEER, 1). Neotenic evolution may explain the lack of the so-called missing links in some cases, and affords a means of emancipation from the restraints of extreme specialization.

---

* The exclusion of most of the Apterygota orders from the Insecta by some authors does not seem to be well justified; this controversy shall be discussed at some length later on. In the meantime, all the orders of the Apterygota are regarded here as belonging to the class Insecta.

The evidence that the insects may have evolved from myriapod-like arthropods by neoteny, is based on the fact that the first instar of many myriapods, such as *Strongylosoma* and *Iulus* in the Diplopoda, show a great number of similarities with the insects, namely, a head of six united segments, a thorax composed of three segments, each with a pair of legs, and an abdomen of about five segments without appendages, or with only very vestigial legs. If a form like this becomes neotenic, undergoing a retardation in the development of the legs behind the first three pairs,* and the larval number of body segments retained into the adult stage, it would be very insect-like and could give rise to the Apterygota. It is interesting to point out that among the Apterygota, there are many species which have leg-like appendages on the abdominal segments. Furthermore, there is a large group, the Collembola, in which the abdomen is composed of six segments only.

Evidence that the myriapods are most closely related to the ancestors of the insects is indicated by the similarity of several characters found in common in the myriapods and insects. These characters are the following:

1) Absence of the dorsal muscle (levator) of the pretarsus in all myriapods and insects.
2) With one pair of antennae. Moreover, antennae composed of a variable number of true segments each being supplied by one or more muscles, is a common feature in all the myriapods and in some primitive insects (Diplura and Collembola).
3) Retention of at least two segmental organs in the head, sometimes only partially retained, is common in some myriapods and primitive insects, in which they function as salivary and nephrocytic organs.
4) The problematic organ of Tömösvary (postantennal organ) is common to Diplopoda, Symphyla and Collembola.
5) Eversible sacs at the bases of the appendages are common to all Symphyla, some Diplopoda, Pauropoda, Diplura, and several lepismid genera.
6) The similarity of the Malpighian and tracheal tubes in both groups.
7) The similarity in structure and development of the heart and aorta. Segmental blood vessels occur in Diplopoda, Chilopoda, and even in the orthopteroid insects.
8) A massive fat body in the haemocoele is common in both groups.
9) Ecdysis takes place through a transverse split at the hind border of the head in myriapods, Protura and Collembola.

As has been already mentioned, among the myriapods, the Symphyla seem the closest to the insects, especially to the primitive forms. This is suggested by the following characters, common to Symphyla and insects:

1) A Y-shaped epicraneal suture or ecdysial suture is common to many Symphyla and insects. However, it should be pointed out, that since the arms of the epicraneal suture vary in the different groups of insects, being merely the lines along

---

* According to Ryuichi Matsuda, in a personal communication, abdominal legs are more conspicuous in embryos of insects, for which reason loss of abdominal legs cannot be derived by neoteny.

which the cuticle splits in molting, and since other sutures have been confused with it, its morphological value is not as great as was once thought.

2) The structure of the postmandibular appendages of the head is essentially the same in Symphyla and Insecta. The first postmandibular appendages are paired maxillae, each is composed of stipes and cardo, the stipes having a pair of apical lobes, the galea and lacinia. This type of maxilla is found only in Symphyla and Insecta. The second postmandibular appendages are united, forming a labium.

3) A distinctly three-lobed hypopharynx, consisting of a median lingua and a pair of anterolateral superlinguae, is found in the Symphyla and some primitive insects, such as Diplura, Collembola, Machilidae, and the larvae of Ephemeroptera (according to George Byers, in a personal communication, some cranefly larvae have also a three-lobed hypopharynx).

4) The head apodemes of the Symphyla are, according to SNODGRASS (35), "suggestive of the anterior arms of the tentorium of Thysanura and pterygote insects, inasmuch as they give attachment to the same muscles that are attached to the insect tentorium."

5) An abdomen with styles and eversible sacs is a common feature of the Symphyla and Diplura.

6) The terminal cerci of Symphyla seem to correspond to those of insects. Furthermore, the cerci of some Symphyla (*Scolopendrella*) and Diplura (*Anajapyx*) are provided with similar spinning glands.

7) A premandibular segmental organ is evident in the late embryo of Symphyla, and in some species it survives in the adult. Evidence of the premandibular organ in the insects was first found in the orthopteran embryos and was called "suboesophageal body." It is also found in Plecoptera, Isoptera, Mallophoga, Coleoptera and Lepidoptera.

8) The peculiar 'dorsal organ' of the embryo of Symphyla is also found in Collembola and *Campodea* and even in higher insects (see JOHANNSEN and BUTT, 20).

9) Embryologically, TIEG, 37, 38) has shown that the Symphyla have fourteen trunk segments, as in insects.

10) To these we may add that, according to MANTON (23), "the full range of insectan gaits (i.e. relative duration of forward and backward strokes and phase differences between successive legs) is seen in the Symphyla, but not in the adult of any other group of myriapods."

The theory that the Symphyla stand closest to the ancestor of the insects is fairly well supported by the above data. Apparently, however, it presents a few weaknesses. According to SNODGRASS (35), those who believe in the Symphyla as the ancestral relatives of the insects "give too little attention to the discrepancy in the mandibular structure." In spite of this TIEGS and MANTON (39) made the following statement, "the mandibles, though segmented, could be the forerunner of the thysanuroid mandible." Another major objection is the progoneate condition of the Symphyla, that is to say, the reproductive organs of these myriapods open upon the third somite behind the head. To this, TIEGS and MANTON (39) replied: "A suggestion that comes to mind is that some myriapods have found it expedient to evolve a new genital opening (one might point, for analogy, to the accessory male genitalia in Odonata) when the terminal segments became involved in anamorphosis and many Diplopoda even show sexual precocity."

Coming back again to the idea of evolution by neoteny, it should be recalled that insects could not have been derived from adult Symphyla, for the

structure of the latter is too specialized. In the same way, neoteny could not have occurred with the type of development which the Symphyla have at the present time, and give origin to the insects. This is due to the fact that the young in Symphyla hatch with six or seven pairs of legs. Therefore, if we assume that the insects originated from the myriapods by neoteny, and at the same time believe in the Symphyla as the ancestral relatives of the insects, we must have to presume that the present development of the Symphyla is secondary, and that formerly the young hatched with three pairs of legs, just as is the case with Diplopoda and Pauropoda. Attention is called to the fact that the first moult of the hexapod larva of Diplopoda gives origin to a young with usually seven pairs of legs, which would correspond to the present stage of the young in Symphyla. This may suggest, perhaps, that the Symphyla have repressed the first stages only.

In summary, our present knowledge seems to indicate that the myriapods, especially the Symphyla, stand closest to the ancestors of the insects. Furthermore, the peculiar hexapod larvae of many myriapods may also suggest that the Insects could have originated by neoteny from myriapod-like arthropods.

MAJOR TRENDS IN THE EVOLUTION OF INSECTS

Three main radiations seem to have taken place during the evolution of the insects. The second and third of these radiations were typically explosive evolution. In insects the first radiation is represented by the Apterygota, found from the Devonian to the Recent. This was the first step in the evolution of insects and therefore the one that had and still preserves the most primitive characters shared only with the myriapods. The second step in the evolution of insects was represented by the Paleoptera, presumably the first insects which acquired wings, but could not flex them on the back of the abdomen. This group is represented in the geological strata of the lower Pennsylvanian to Recent. The acquisition of wings made them a dominant and successful group. The Paleoptera seem to have undergone an explosive evolution which somewhat paralleled the Neoptera. Thus we find forms which were similar to Hemiptera (Protohemiptera*), others were like the Hymenoptera (Protohymenoptera), and still other forms acquired independently the capacity to flex the wings (Megasecoptera), etc. The Paleoptera were very abundant during the Pennsylvanian, much more so than the Neoptera of that time; but after the Permian they diminished in number, displaced by the better adapted Neoptera and today this primitive group is represented only by two orders, the Ephemeroptera and Odonata. The third and last radiation of insects was the Neoptera, presumably derived from the Paleoptera. They were characterized by the capacity to flex the wings. This characteristic was obviously more advantageous than the inability to fold them. It permitted the insect to occupy more ecological niches

---

* For a general discussion of the extinct orders see CARPENTER (2).

which otherwise could not be occupied since paleopteroid wings were a hindrance for the insects in places such as water, underground, brushy areas, etc. The Neoptera are found in the geological strata from the lower Pennsylvanian to Recent, and have radiated into the numerous phyletic lines of our modern insects.

THE APTERYGOTA

It is the contention of some authors that the entognathous groups (Collembola, Protura, Diplura) should be excluded from the insects. This has been based on the following unusual features found in these groups:

UNUSUAL FEATURES OF PROTURA

1) Lack of antennae.
2) The mandible is articulated to the cranium by a slender rod, as in Chilopoda. It should be pointed out, however, that this rod was not observed by TUXEN (43). He adds further that "perhaps it is the fanlike ligament taking this aspect in certain views."
3) The abdomen is composed of twelve segments, one segment too many.
4) The gonopores of both sexes are found on the eleventh abdominal segment. It should be recalled that in all insects, excepting Protura, Collembola, and Ephemeroptera, the gonopores are found on the eighth for the female and on the ninth for the male, although some females have the gonopores open on the ninth also. The gonopore in both sexes of Protura is surrounded by small external genitalia of an unusual form, comparable to that of no other group.
5) The Malpighian tubes are represented by six papillae.
6) The inmature instars have a myriapod-like development. They develop by anamorphosis, that is, when they hatch they have nine abdominal segments, the other segments are added during the postembryonic growth, which involves five larval stages.

UNUSUAL FEATURES OF COLLEMBOLA

1) Flagellar muscles in the antennae.
2) The mandible is also articulated to the cranium by a slender rod, as in Chilopoda. However, according to TUXEN (43), this is not a real rod but a ligament and therefore not homologous with the rod of Chilopoda.
3) The abdomen is composed of only six segments.
4) The gonopores of both sexes are found on the posterior margin of the fifth abdominal segment.
5) Malpighian tubes lacking.
6) Just behind the antennae of some Collembola (most Poduroidea and some Entomobryoidea) there is a peculiar structure known as the postantennal organ, possibly a sensory organ homologous to the organ of Tömösvary in Diplopoda and Symphyla.
7) Gonads with a lateral germarium; that is, unlike other insects, the germarium of the ovaries and testes is lateral and not apical in position.
8) The egg is holoblastic, as in Symphyla and some diplopods, that is, a total cleavage takes place in the embryonic development, instead of the usual meroblastic and centrolecithal cleavages.
9) Lack of embryonic membranes, that is, the egg does not develop an amnion and serosa, as in the myriapods.

Unusual features of Diplura

1) Flagellar muscles in the antennae.
2) Segmental ovarioles in some species.
3) Lack of embryonic membranes.
4) The Malpighian tubes are represented in some species by small papillae.

It should be pointed out, however, that the entognathous groups stand at least near the base of the phylogenetic tree of the more typical insects (Fig. 1). This would explain such unusual primitive features as the antennae with the flagellar segments provided with muscles; the presence of the postantennal organ; the mandible articulated to the cranium by a slender rod; the abdomen composed of twelve segments, which agrees with the primitive number found in embryonic insects; malpighian tubes represented by papillae; anamorphosis; segmental ovarioles; total egg-cleavage; and even perhaps the lack of embryonic membranes, although this could be a secondary feature, as is the case of some ants in which the amnion is wholly lacking and the serosa is rudimentary, represented by only a few cells. The other unusual characters are mere specializations. Thus, the absence of antennae in Protura is probably a secondary feature; after all, these organs are reduced to minute papillae in many insect larvae. According to Tuxen (42), the pseudoculi are remnants of the antennae, a conclusion reached after investigating their structure, innervation and musculature. On the other hand, the entognathous groups have also typical insectan features. Their mouth-parts are insectan in character; their differentiated thorax and three pairs of legs are also insectan features, as well as the reduction of the abdominal appendages. To this we may add their ontogeny, which is in general insectan in character.

Those who believe that the entognathous groups should be excluded from the insects assume that the Apterygota are a polyphyletic assemblage of arthropods. However, it should be pointed out that the entognathous apterygotes (Diplura, Protura, Collembola) appear to be related, as will be shown later on, to each other. Therefore, if polyphyly has in fact occurred in the Apterygota we should regard the Insecta as a diphyletic group. That this is actually the case we are not sure, but even if true, this would not constitute a satisfactory reason for restricting the definition of the class Insecta so as to exclude them. It has been argued that all the animals within a given group should have had a common origin, that is to say, to be monophyletic. However, we should remember that classification is, after all, a practical problem, and the idea that a group must be derived from a single species of a preceding group, cannot be always met in practice. To begin with, knowledge is far from complete and monophyly certainly cannot be demonstrated in any case. Furthermore, if theoretical monophyly is too strictly demanded, this may give as a result a classification in which the groups (with different names) cannot be distinguished morphologically. For this reason, Simpson (32) has suggested that a group, whatever its rank, should be composed of related animals that can be defined by morphological and related data, and have originated from a group of animals

of lower rank than itself. That is to say, if the group in question corresponds to a class, it should have originated from a unified group smaller than a class, i. e. a superfamily, family, genus, etc. In closing this discussion, one should remember that the class Mammalia is regarded by most paleontologists and mammalogists as a polyphyletic group, and yet, most agree that is should be kept as a class.

It is obvious that the present apterygotes are too specialized to be direct descendants from existing myriapod-like arthropods. There have been several attempts to show which order is the most primitive. According to CRAMPTON (5), the *Machilis*-like Apterygota are morphologically the most primitive, but according to EWING (11), the Protura and Collembola "are to be regarded as groups of very ancient origin." On the other hand, IMMS (18), regards Diplura as the closest relatives of the Symphyla. This diversity of opinions shows very clearly how the primitive characters together with the specialized ones, are diversely distributed among these orders. In other words, the machiloid and the entognathous apterygotes should be considered as a whole as the most primitive insects, or closest to the ancestral group. Therefore, if the insects have any connection with the myriapods, this connection is not to be found in any one of the apterygote orders but rather in all these groups.

The Diplura, Protura, and Collembola seem to be related to each other, as suggested by SNODGRASS (35) and TUXEN (43). That these three orders are related is indicated by the following characters:

1) The mandibles and maxillae are enclosed in pouches of the head wall. This condition is called entognathy; it comes about through the formation of two folds (plicae orales), one on each side of the head, uniting at or below the sides of the labium. Entognathy is found in no other arthropod group.
2) The structure of the mouth-parts similar, as shown by TUXEN (43). Thus "the mandibles of the Diplura and Collembola are rather closely alike both in themselves and as regards their muscles, whereas the mandibles of Protura have a different musculature, are not hollow, and have no teeth. The mandibles of Protura are piercing organs necessitating especially strong protracting and retracting movements; and this, in connection with the probably secondary fact that they are not hollow, may account for the difference... The general plan of the maxilla in the three groups is very much alike; especially the shape and position of the cardo are identical... In all three groups the lacinia carries an arm or platelike process to which one or more muscles are attached, running to the stipes, to the hind wall of the head, or to both. And in all three groups powerful muscles connect the stipes and the distal part of the cardo, as it seems, to the fulcrum."
3) Myriapod type antennae, that is, composed of true segments, each with one or more muscles.
4) The endoskeletal head structure is superficially very similar to the simple myriapodan tentorial arm. According to SNODGRASS (35), however, this tentorium does not correspond to that of myriapods, Thysanura, and Pterygota, but rather to the postoral sternal brachia of Crustacea. FOLSON (12) and HANSEN (13) have shown that the supposed anterior tentorial arms of the entognathous apterygotes are superficial sclerotizations of the sternal wall of the head, rather than true apodemes as in the Symphyla and other myriapods. In Diplura the anterior parts of these brachia are connected inside the head by a ligamentous bridge which seems to correspond to that of Chilopoda. In Collembola this ligamentous

bridge is elaborated into a complicated framework, which according to SNODGRASS (35) has not been demonstrated to be a cuticular structure and is similar to the endosternum of Arachnida. In Protura the anterior parts of the brachia are united, and no ligamentous bridge is found. It has also anterior connections with the cranium which may be secondary apodemal processes.

5) The maxillae are articulated with the posterior parts of the endoskeletal head structure. This, according to HANSEN (13), is a further proof that this type of tentorium is not homologous with that of other arthropods because "in no other arthropod are the maxillae articulated to any internal part of a tentorium."

6) True tarsomeres lacking.

Therefore, the Diplura, Protura, and Collembola, seem to have evolved at first in the same direction, but then they followed different lines (Fig. 1). It appears, then, that these three groups are better represented in a phylogenetic tree as on the same plane or level after splitting up from a common branch. The Protura and Collembola are more specialized in many ways than the Diplura, although they retain several primitive characters not found in Diplura. The Diplura is the most insect-like of the three and perhaps the least specialized.

The remaining groups of Apterygota are the lepismatids and machilids, which are usually regarded as forming the order Thysanura. However, they are separated here in two orders or phyletic lines, as has already been done by CRAMPTON (6), HENNING (14) and ROSS (29). There is no doubt that these two groups are similar in their general appearancce, but on the other hand, they differ structurally on several important features. These differences are summarized in table 1.

TABLE 1

Differences between Lepismatids and Machilids

Machilidae	Lepismatidae
Tentorium very primitive, the anterior tentorial arms similar to the head apodemes of the myriapods	Tentorium approaches the orthopteroid type in that the anterior arms are usually confluent in a large central plate
Mandibles with a single articulation	Mandibles are doubly articulated
The machilid jaw is the most primitive mandible found among the insects; they are almost crustacean in musculature	The musculature of the mandible is similar to that of all pterygote insects in which the ventral adductors are retained
The hypopharynx is a three-lobed structure, as in the Diplura and Symphyla	The hypopharynx has attained the development typical of the lower pterygotes

The machilids and lepismatids are in fact so different in fundamental features that each has been given ordinal or subordinal rank since the beginning of this century. The oldest names given to the machilids are as follows:

Machiloidea by Handlirsch, 1903 - as an order
Microcoryphia by Verhoeff, 1904 (April 22) - as an order
Archaeognatha by Börner, 1904 (May 3) - as a suborder

Since Machiloidea has been used as a superfamily name (REMINGTON, 27), Microcoryphia seems the proper name to use for the order. The old name Thysanura should be retained for the lepismatids.

Although the Microcoryphia show no specialization of the mouth-parts like the entognathous apterygotes, they are a little higher in relative advancement, as indicated by the following characters:

1) Development without any of the primitive features of the entognathous apterygotes, namely without anamorphosis, without total cleavage, without dorsal organs in the developing egg; and with embryonic membranes.
2) Flagellar segments not musculated.
3) Tentorium with anterior, posterior, and dorsal arms, comparable to those of lepismatids and pterygotes. However, it is not fused into a large central plate. It should be pointed out that the anterior arms of the tentorium in machilids and lepismatids have a ventral position on the head.
4) Genitalia comparable to that of other pterygotes.
5) True tarsomeres present.

Although the Microcoryphia stand a little higher in the trunk of the evolutionary tree, they are not too far from the entognathous apterygotes, as is shown by the few similarities with the Diplura, namely a three-lobed hypopharynx, cerci, styli, and eversible sacs. On the basis of these resemblances the Diplura have been placed among the Thysanura but this arrangement does not fit the information discussed above.

Of all the apterygotes, the Thysanura (s. str.) stand highest in the evolutionary tree, and therefore closest to the pterygote insects. That they are less primitive than the Microcoryphia has been shown already in Table 1.

In summary, the Apterygota are composed of five main groups of insects: Diplura, Protura, Collembola, Microcoryphia, and Thysanura. The first three show a few features in common which may indicate certain interrelationships among them. Although the Diplura, Protura and Collembola are groups of very ancient origin, they should be regarded as insects judging by the many characteristics which they share with other apterygotes and with the more generalized pterygotes. The Microcoryphia and Thysanura are more insect-like, but they still retain primitive features and should be regarded as ancestral groups; they have styli on the thoracic legs; appendages on the abdominal segments; labial kidneys; a short germ band; an open amniotic cavity; an amnion developed by invagination; and a medial frontal organ with double cells and a disk-shaped structure, as in Crustacea. On the other hand, as MARCUS (24) has already indicated, they "also show characters in common with the Pterygota: mouth-parts; distinct paranota on all segments; antenna divided into shaft and flagellum and provided with Johnston's organ; a typical number and position of the stigmata; and an orthopteroid ovipositor formed of the eighth and ninth abdominal legs."

THE PTERYGOTA

The first major explosive evolution of the insects began with the development of wings. This ability to fly changed the direction of insect evolution. The actual derivation of the pterygotes from the apterygotes has been the subject of much speculation.

There are three main theories as to the origin of the wings. All these views agree in that the wings were developed from paranotal processes on the meso-and meta-thorax. This is based on the fact that many of the fossil insects (Palaeodictyoptera) show these processes on the prothorax and sometimes on the abdomen.

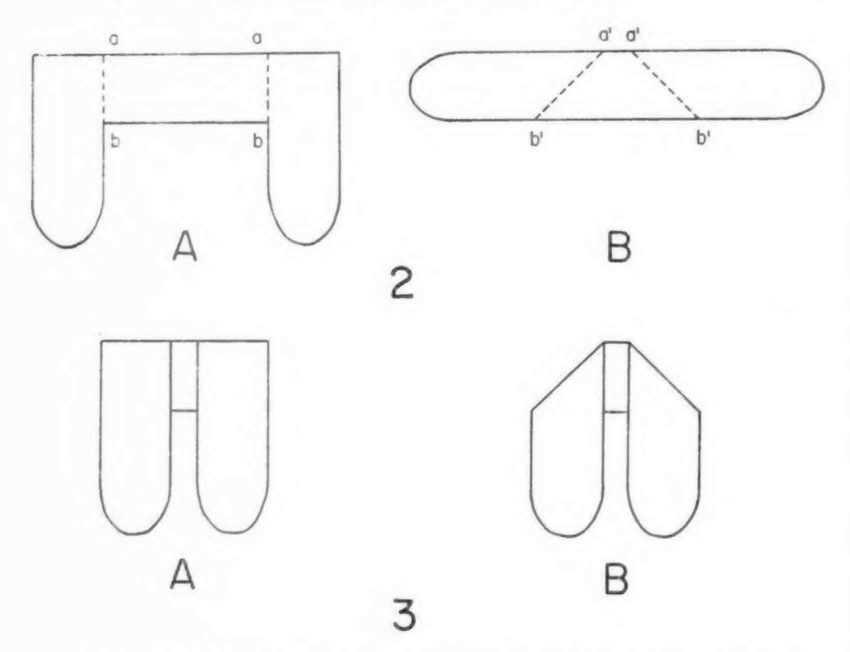
The classic view is that of MARTYNOV *et al.* (25). According to them the paranotal extensions tended to grow sidewards and were used as gliding organs. Since these processes had a selective value they were enlarged further. This happened only with the meso-and metathoracic extensions, probably by a series of mutations fixed by natural selection. The next step was the acquisition of a line of flexibility at the base of each process. In this way the wings originated. These wings were at first paddling wings, but later some acquired the ability to flex them on the back of the abdomen, thus giving origin to the neopterous insects. According to this view the insects are naturally divided into two groups: The Paleoptera, including those insects incapable of flexing the wings (primitive condition), such as Odonata and Ephemeroptera, plus the fossil groups Palaeodictyoptera, Protohemiptera, Protodonata, Protoephemeroptera, and Megasecoptera; and the Neoptera, including those insects capable of flexing the wings on the back of the body (secondary condition), such as the rest of the insects.

SCHWANWITSCH (31) points out that an insect with immobile gliding paranota would be hindered in its crawling through the vegetation. He believes that the paranota were directed backwards and became mobile from the very beginning of their evolution. In this way they could help walking by their strokes as in the case of *Bombyx mori*, which has lost its flight. Later they were transformed into true wings by elongation. Therefore SCHWANWITSCH'S view is the opposite of that of MARTYNOV, because he assumes that the ability to flex the wings on the back of the abdomen should be regarded as primitive and the spread out position as secondary. He divides the Pterygota into two main types, based on the wing motor musculature. The first type is found in Odonata, which have two systems of dorsoventral muscles for the up and down movements of the wings; this he named Orthomyaria. The other type is found in all the rest of insects in which the upward movement of the wings is accomplished by dorsoventral muscles as in Odonata, but the downward movement is achieved by longitudinal muscles, this he named Chiastomyaria (SCHWANWITSCH, 30).

The third view is somewhat intermediate to the two previous ones in that both conditions, flexed and unflexed, are considered primitive. LEMCHE (22) believes that the wings originated from two different types of growth of the paranotal process. In one the growth was sidewards giving rise to the

Palaeodictyoptera, Protohemiptera, Protodonata, Odonata, and Megasecoptera. In the other, growth was backwards and gave origin to the rest of the orders. And these two lines of development were independent of each other. LEMCHE based this belief on the following observations.

1) The fossil record does not contradict the diphyletic conception of the Pterygota. Both groups, Paleoptera and Neoptera, are found at the same time from the lower Pennsylvanian onward.
2) All the known nymphs of the Palaeodictyoptera had a sideward growth of wing pads. On the other hand, the nymphs of Protorthoptera and Blattaria had wing pads directed backward.
3) All present-day Pterygota develop the wing pads backwards at least in all the groups in which this growth can be observed, with the exception of the Saltatoria and Odonata. In the Saltatoria, however, the wing pads start growing backwards just like those of Blattaria, but in the next molt these wing pads are titled along axes parallelling the longitudinal axis of the body, thus showing a secondary condition. It is comparable to Blattaria. In the Odonata, the wings start growing as small vertical ridges at the sides of the meso-and metathorax. These ridges grow from lines forming an angle with the longitudinal axis of the body. At the same time the wings are tilted as in Saltatoria, therefore the growth of the wings in Odonata is essentially a sideward growth; on the other hand, in the Saltatoria the growth is backward. This is best illustrated by cutting two papers as shown in figure 2 and folding them along the lines marked a-b, a'-b'.



The paper models then appear with the flaps directed backwards (Fig. 3). Actually when they are unfolded only model A (which represents Saltatoria) has backward projections, model B (which represents Odonata) has sideward extensions.

4) The tracheal ramifications in the wings that grow backwards show that the wing tracheae are bent toward the back from the very beginning, displaying the same relation to the hind corner of the paranotum as the veins in the full grown wings to the wing tip. The tracheal ramifications in the prothorax are quite similar. This seems to indicate that the wings have not changed secondarily to the backward growth. Thus the tracheae were originally directed backwards, parallelling the longitudinal direction of the wing or spreading out in the shape of a fan. On the other hand, in the Palaeodictyoptera where the wings grew sidewards, the tracheal ramifications or veins must have run in curves toward the posterior edge. In fact, the Palaeodictyoptera do show the veins extended in even arches toward the posterior edge, and the subcosta tends to end farther out in the wing.
5) On the basis of wing growth type, the Pterygota can be divided into two groups: The Plagioptera, including those with lateral wing pads, such as Palaeodictyoptera, Protohemiptera, Megasecoptera, Protodonata, and Odonata; and the Opisthoptera, including those with posteriorly directed wing pads, the rest of the orders. On this criterion the Ephemeroptera and Odonata are placed on different lines, and not in the same group, as in the division into Paleoptera and Neoptera. Here LEMCHE finds support on the morphology of these two groups. The Ephemeroptera actually show a thoracic musculature and wing base comparable to those of the Neoptera (MATSUDA, 26). On the other hand the Odonata show a wing base quite different from any other living insect. They have, instead of the epipleural and axillary sclerites, two large plates. Also the thoracic musculature of Odonata is different from any other insect; it is composed mainly of dorsoventral muscles, which perform the depression and elevation of the wings. The depressors of the wing are dorsoventral muscles inserted on the two large plates. The pleural wing process has two arms, each one supporting one of the large plates of the wing base. The depression and elevation of the wings in Odonata was solved, according to SNODGRASS (36), as follows: "Since the dorsal walls of the paranotal lobes or the wing bases are lateral extensions of the notum, muscles here attached have simply retained their original notal connections. The problem of the early dragonflies then was to divide these muscles into two functional groups pulling on opposite sides of the wing fulcrum. The problem was readily solved by curving the wing fulcrum inward until it supported the wing base between lateral and mesal groups of the muscles (fig. 23B). The muscles attached mesad of the fulcrum thus became wing elevators (B) and those laterad of it became wing depressors (C)." In all other insects the depression of the wings is performed by longitudinal muscles, instead of dorsoventral muscles as in Odonata. The flexion of the wing is possible by the position of epipleural and axillary sclerites. The flexor of the wing is a muscle (or muscles) inserted on the third axillary sclerite; therefore it is present in all the wing-flexing insects (in Odonata, a muscle from the pleural ridge to the posterior or axillary plate may correspond to the third axillary muscle of other insects). The extension of the wing is accomplished mainly by the basalar muscles. The main reason why the Ephemeroptera cannot flex the wings is that the muscle of the third axillary sclerite is absent (according to MATSUDA, a muscle from the pleural ridge to the second axillary, in Ephemeroptera, probably corresponds to the muscle of the third axillary). It should be pointed out also that according to HENRIKSEN (15), the Odonata molt in a way somewhat different from any other insect. The usual manner is a splitting of the skin along the middle of the tergum of the thorax. In Odonata this middle splitting of the thorax is confined only to the prothorax, and anterior portion of the mesothorax, the ecdysial line then branches toward the bases of the wings and runs backwards and outwards on each side.

Of all the living groups of Pterygota the Ephemeroptera seem to be

the most primitive. This, of course, does not mean that the ephemerids are not specialized in any way. On the contrary, every living group or form tends to show a combination of primitive and specialized characters, often to such an extent that it may disconcert the most expert phylogenist. Nevertheless, the balance of these characters, and what is still more significant, the importance of these features which are considered primitive or specialized, will determine more or less the position of the group under consideration. To determine the importance of a character in a group it must be compared with those which are believed to be more primitive and more advanced. The primitive characters of the Ephemeroptera are best seen in the nymphs. They are the following:

1) The hypopharynx is a three-lobed structure, as in the Symphyla, Diplura, Collembola, and Microcoryphia (*Machilis*).
2) The mandibles with only a single articulation, as in *Machilis*.
3) Tentorium resembling that of *Lepisma*, with a broad central plate and four supporting arms; the anterior arms are likewise ventral in position as in Thysanura.
4) The nymphal gills of ephemerids have been homologized with the abdominal styli of Thysanura. However, there is not enough proof of their homology but the concept is reasonable and not too improbable.
5) Apical appendages of the abdomen (cerci and caudal filaments) similar to those of Thysanura.

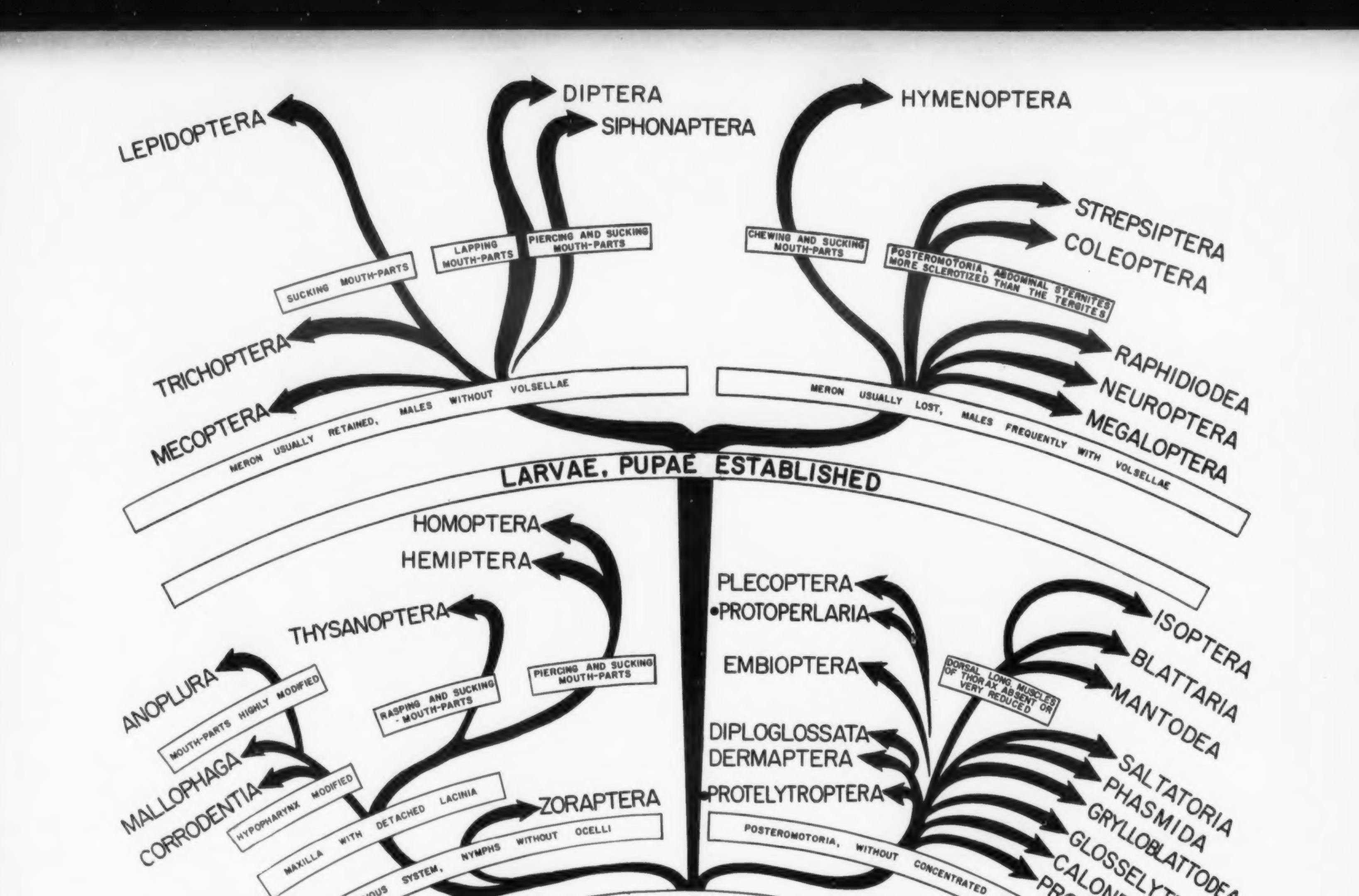
The adult mayfly also presents some primitive characters:

1) The wing-venation of these forms is considered to be the most primitive of all living winged insects by TILLYARD (40) and EDMUNDS and TRAVER (10). This is based on the fact that the wing approaches the hypothetical archetype wing-venation (COMSTOCK, 3, and SNODGRASS, 33); with intercalary veins and a fluted condition. The last two features have been regarded by several workers as specializations rather than primitive characters. However EDMUNDS and TRAVER point out that probably the first winged insect had a completely fluted wing, with alternation of concave and convex veins. This was very important to strengthen the wing and necessary before the wing could become thin. Such thinning of the wing was a requisite to improve the sculling action of flight, and constitutes a trend found in most insects in which the wings are rigid along the anterior margin and membranous behind.
2) The Ephemeroptera are the only Pterygota that undergo a molt after reaching the state of imago. This seems to indicate a holdover from the Apterygota ancestors which molt periodically throughout life.
3) The male has paired gonopores with corresponding double penis. The female possesses also paired gonopores which are rather unique among insects; these gonopores are found on the seventh abdominal segment.
4) Thysanura-like ovaries.

The Odonata are an isolated group, a single side branch in the phylogenetic tree; this is also true of the Ephemeroptera. The features which make the dragon-flies an isolated group have been described. However, among all the living orders of Pterygota the Ephemeroptera are the closest to them. This fact is indicated by the following characters that they have in common.

1) Inability to flex the wing on the back of the abdomen, although morphologically speaking this common character may lack any significance.





LEPIDOPTERA
DIPTERA
SIPHONAPTERA
HYMENOPTERA
STREPSIPTERA
COLEOPTERA
SUCKING MOUTH-PARTS
LAPPING MOUTH-PARTS
PIERCING AND SUCKING MOUTH-PARTS
CHEWING AND SUCKING MOUTH-PARTS
POSTEROMOTORIA, ABDOMINAL STERNITES MORE SCLEROTIZED THAN THE TERGITES
TRICHOPTERA
MECOPTERA
RAPHIDIODEA
NEUROPTERA
MEGALOPTERA
MERON USUALLY RETAINED, MALES WITHOUT VOLSELLAE
MERON USUALLY LOST, MALES FREQUENTLY WITH VOLSELLAE
LARVAE, PUPAE ESTABLISHED
HOMOPTERA
HEMIPTERA
THYSANOPTERA
PLECOPTERA
•PROTOPERLARIA
EMBIOPTERA
ISOPTERA
BLATTARIA
MANTODEA
DORSAL LONG MUSCLES OF THORAX ABSENT OR VERY REDUCED
ANOPLURA
MOUTH-PARTS HIGHLY MODIFIED
RASPING AND SUCKING MOUTH-PARTS
PIERCING AND SUCKING MOUTH-PARTS
MALLOPHAGA
CORRODENTIA
HYPOPHARYNX MODIFIED
MAXILLA WITH DETACHED LACINIA
ZORAPTERA
NYMPHS WITHOUT OCELLI
DIPLOGLOSSATA
DERMAPTERA
PROTELYTROPTERA
POSTEROMOTORIA, WITHOUT CONCENTRATED
SALTATORIA
PHASMIDA
GRYLLOBLATTODEA

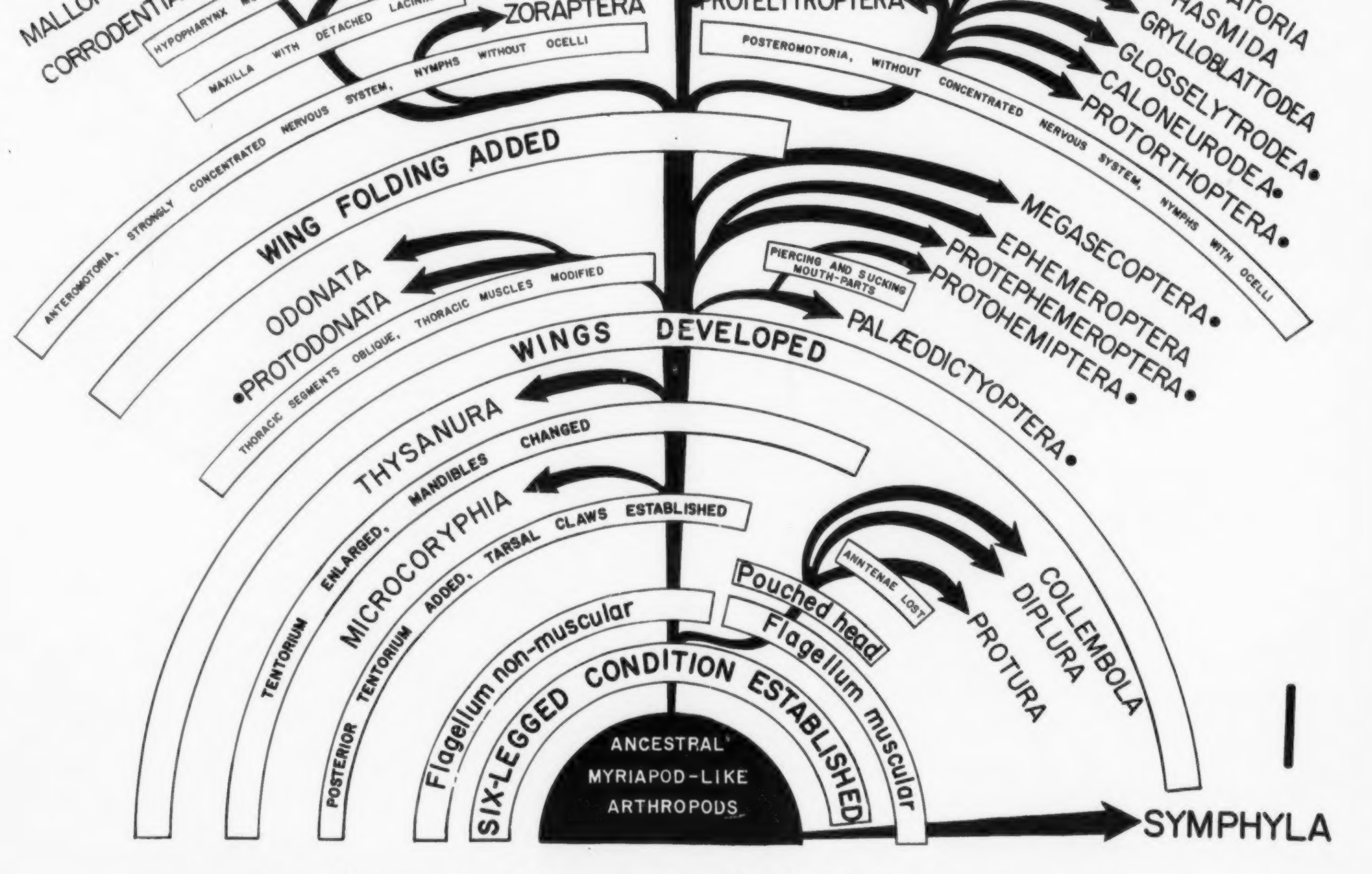


Fig. 1: Phylogenetic tree showing current interpretation of relationships of the insect orders.



TABLE 2

The Three Main Evolutionary Trends of Neoptera

Orthopteroid	Hemipteroid	Neuropteroid
Metamorphosis simple Nymphs with ocelli	Metamorphosis simple Nymphs without ocelli (According to Dr. H. B. Hungerford* some gelastocorid nymphs have ocelli)	Metamorphosis complete
Without tendency to develop sucking mouth-parts. All of them (except some termites) lack a well developed gular region	With gradual development of sucking mouth-parts The maxillae with a detached portion (possibly the lacinia) forming a chisel like structure	With tendency toward the development of proboscis. Some with well developed gular region and tendency to form a hypostomal bridge
Antennae normally long and multiarticulated, reduced only in the more specialized forms	Tendency toward reduction of the antennae	Antennae usually filiform
Posteromotoria, that is, the hind wing and its musculature predominate	Anteromotoria, that is, the fore wing and its musculature predominate	Anteromotoria, except Coleoptera in which the hind wing and its musculature predominate
Usually with well developed anal fan in the hind wing	Without dilated anal area in the hind wing	Usually without dilated anal fan in the hind wing
With well developed basisternum.	Poorly developed basisternum. Some with descrimen (Homoptera).	Without exposed basisternum. With descrimen
Tarsomeres variable	Never more than three tarsomeres	Usually with five tarsomeres
Cerci well developed	Without cerci, except Zoraptera	Cerci may be present (Mecoptera, Siphonaptera)
Males with styli and females with well developed ovipositor (lacking in Plecoptera, Embioptera, Gryllotalpidae and most Dermaptera and Isoptera).	Males usually with styli. Some females with well developed ovipositor: Thysanoptera, Homoptera	Males with styli. A well developed ovipositor found in Hymenoptera
Mesotrochantin usually prominent	Mesotrochantin tends to become slender and its base fuses with the mesopleura	Mesotrochantin fused to mesopleura
Paraprocts usually distinct	Paraprocts usually indistinct or wanting	Paraprocts indictinct or wanting
Numerous malphigian tubes (exceptionally reduced in some termites)	Few malphigian tubes, not more than six, generally four	Few malphigian tubes, usually reduced to six
Without concentrated nervous system	Strongly concentrated nervous system	Usually without concentrated nervous system (but strongly condensed in some Diptera).

* Personal communication.

2) A rather primitive wing venation, conserving the anterior media, which in most modern insects has been lost. In Ephemeroptera both branches of the media are retained (MA and MP), in Odonata only the primitive anterior media is retained. The wing shows more or less a regular alternation of convex and concave veins, at least in the sub-basal region. Anal veins tend to curve toward the rear.
3) Malphighian tubes numerous.

It should be remembered, however, that the Ephemeroptera and the Odonata are not related insects. As SNODGRASS (36) has indicated "they represent two early lines of pterygote evolution differentiated by the method adopted for moving the wings."

The second major explosive evolution of the insects began with the capacity to flex the wings, and is represented today by the so-called Neoptera. In spite of the great diversity of the neopterous orders they can be characterized as follows:

1) Ability to fold the wings on the back of the abdomen, as a result of the presence of the muscle of the third axillary sclerite.
2) A new region in the wing is developed, the jugal region or neala.
3) The anterior media is usually lost, thus leaving the entire spaces between R_1 and Cu, with only concave veins. Anal veins are directed obliquely toward the posterior margin of the wing.

The Neoptera are represented by three main evolutionary trends or groups: The orthopteroid, hemipteroid, and neuropteroid. MARTYNOV named them, according to the evolution of the jugal region or neala, Polyneoptera (orthopteroid) with well developed neala containing several veins; Paraneoptera (hemipteroid) with only a simple or a branched vein in the jugal region and Oligoneoptera (neuropteroid) with a simple and longitudinal vein in the neala. The characters of these groups are tabulated in table 2.

The characters indicated are trends rather than clear cut features. Some of the exceptions are characters secondarily lost or primitively retained.

TABLE 3

The Two Major Lines of the Orthopteroid Group

Orthopteroid Proper	Panplecoptera
Ovipositor well developed	Ovipositor wanting or greatly reduced
Forewings usually chitinized, used to protect the hindwings	Forewings usually membranous
Usually with five tarsomeres (variable in Saltatoria)	Three tarsomeres

THE ORTHOPTEROID GROUP

In this group we can distinguish two lines: the orthopteroids proper, with clear affinities, and a line represented by Plecoptera, Embioptera and Dermaptera, with doubtful affinities (although Dermaptera be closer to the typical orthopteroids). The distinguishing trends of these two lines are indicated in table 3.

In the orthopteroids proper we find two distinct lines: the Panorthoptera, represented by Grylloblattodea, Saltatoria, and Phasmida. The Dictyoptera, represented by Blattaria, Mantodea and Isoptera. The distinguishing characteristics of these two lines are indicated in table 4.

TABLE 4

The Two Evolutionary Lines of the Orthopteroid Proper

Panorthoptera	Dictyoptera
Ovipositor usually well developed and basally overlapped by the eighth abdominal sternite	Ovipositor reduced, no longer serving for egg-laying, and basally overlapped by the seventh abdominal sternite
Eggs not laid in an ootheca	Eggs laid in an ootheca
Male genitalia symmetrical (except for *Grylloblatta*)	Male genitalia asymmetrical and complex
Pronotum not tending to have lateral lobes	Pronotum tending to have lateral lobes
Lateral cervical sclerites not tending to be contiguous in the midventral line	Lateral cervical sclerites tending to be contiguous in the midventral line
Coxae relatively small	Coxae very large
Cerci sometimes with mesal prongs and ordinarily short and non-segmented (except in *Grylloblatta*)	Cerci multiarticulated

An interesting fact, as pointed out by SNODGRASS (36), is that the dorsal longitudinal muscles of the thorax, in the Dictyoptera, "are either absent or are too small to have any direct action on the wings. "For which reason these insects also make use of the dorsoventral muscles for wing motors, but not in the same way as do the dragonflies." The method by which the Dictyoptera move their wings is not well understood. To all these, Snodgrass adds that the

Dictyoptera, "on the basis of their wing musculature and thoracic structure, are appropriately separated in classification from the rest of the orthopteroid insects."

Although the termites superficially seem to be far apart from the roaches, there is little doubt that they arose from roach-like insects. A close examination will reveal the true relation of these two groups ,Table 5).

TABLE 5

Similarities Between Roaches and Termites

Roaches	Termites
Five tarsomeres	Although the usual number of tarsomeres is four, the primitive family Mastotermitidae is characterized by having five tarsomeres
Anal lobe in the hind-wing	Here again the great majority lack the anal lobe, but is present in the family Mastotermitidae
Reduced ovipositor	Although in most termites the ovipositor is completely absent, in Mastotermitidae it is present and reduced as in the roaches
Eggs laid in rows and in an ootheca	The dropping of single eggs is the common habit of most termites, but in Mastotermitidae we find that they lay the eggs in masses cemented together in two rows, thus simulating oothecae
The roaches are not social. Nevertheless some subsocial roaches are surprisingly termite like in habits, like the wood-boring roach *Cryptocercus punctulatus*. This species lives in colonies which are composed of parents and offspring, and like the termites, feeds on wood	Always social
Also like the termites, *Cryptocercus* harbors wood-digesting protozoa in its alimentary tract. Some of these protozoa belong to groups that occur in termites	Symbiotic cellulose-digesting protozoa in their alimentary tract
The Australian roach *Panesthia*, a soilburrowing blattid, breaks off its wings just as winged termites do	Winged termites break off their wings

To all these characters in common it can be added that according to JUDD (21), the structure of the proventriculus in both groups indicates a close relationship.

Regarding the Panplecoptera, CRAMPTON (6) has summarized the evidence that the Embioptera and Plecoptera are related as follows:

1) Mesothoracic postscutellum well developed.
2) Mesotrochantin basally fused with the pleuron.
3) Mesothoracic coxae tend to become ring-like.
4) Three tarsomeres.
5) Ovipositor lacking.

THE HEMIPTEROID GROUP

The Zoraptera are placed here as the most primitive group of the hemipteroids, at the base of their evolutionary branch and close to the orthopteroids. The special position of Zoraptera is shown by the multiple affinities with the hemipteroids and orthopteroids. DELAMARE-DEBOUTTEVILLE (8, 9) has indicated remarkable similarities between Zoraptera and Isoptera (the lateropleural region of wingless forms as compared with the winged forms in both groups, also the resemblance of the propleuron in both orders, plus the ability of the winged forms to shed the wings).

The main evolutionary trend in the hemipteroids is a gradual development of sucking mouth-parts. The Zoraptera present a conservative type of mouth-parts with only slightly elongated maxillae. In Corrodentia the maxillae present a peculiar modification, the lacinia seems to have detached completely from the stipes, forming a chisel-like rod, toothed or forked at its apex. This rod is provided with a protractor muscle from the stipes of the maxilla and a retractor muscle from the head wall. Another important modification is found in the hypopharynx. The lingual sclerites (basal bars) of the typical hypopharynx have been transformed into a pair of large sclerites of ovoid shape. The suspensory sclerites and the sitophore have been modified also into a conspicuous cup-shaped sclerite, placed just before the mouth. These two structures are connected by a branched filament. The ovoid sclerites have been regarded as glands, and the filament as a duct. This concept has been proved to be wrong, as WEBER (45) and others have shown conclusively that the sclerites in question do not have a glandular structure in the epithelium beneath them, and are devoid of lumen. According to WEBER (45) there is a small and hard process arising from the clypeal wall of the preoral cavity which fits into the cup-shaped sclerite thus forming a "mortar-and-pestle" apparatus (v. SNODGRASS, 34, p. 25). These morphological changes are also found in the Mallophaga: a rodlike lacinia free from the maxilla, with the corresponding retractor muscle from the head wall, and protractor fibers from the stipes. This rod, however, has been lost in many Mallophaga. The ligular sclerites and the suspensory sclerites, including the sitophore, have undergone the same transformation as in Corrodentia. The function of the sitophore sclerite in Mallophaga

is unknown, an opposing "pestle" as in Corrodentia being absent in this order

The Anoplura are a specialized side branch of the Mallophaga, in which the mouth-parts have been highly modified. In Thysanoptera we already find a short and conical beak with three styli. One stylet is the transformed left mandible, the right mandible is absent or very reduced. The other two styli correspond to the maxillary lacinia. REYNE (28) has shown that the maxillary stylet of the Thysanoptera is developed from a secondarily detached part of the maxilla. Thus, the maxillary styli would correspond to the chisel-like rods of Corrodentia and Mallophaga, that have become elongated and setiform. In some forms the maxillary stylet is connected with the base of the stipes by a lever arm. According to SNODGRASS (34) this lever arm belongs to the lacinia. No lever has been differentiated in Corrodentia and Mallophaga. The maxillary stipes and galea are placed at the sides of the conical beak.

In Hemiptera-Homoptera the beak reaches the highest development. The maxillary structure of Thysanoptera is again found in this group. This is best seen in cicadas, in which the maxillary stylet is connected with the stipes by a lever sclerite, just as in Thysanoptera (v. SNODGRASS, 34, p. 97). The main difference consists in that the maxillary lobe has become fused with the lateral wall of the head and the maxillary palpus is absent.

THE NEUROPTEROID GROUP

Like the Hemipteroids, the Neuropteroids probably descended from forms closely allied to Protorthoptera. Therefore it is not surprising to find that some forms (Coleoptera) are very orthopteroid in several features. As a matter of fact, WEBER (46) includes the Coleoptera among the orthopteroids. The complicated interrelationships among the neuropteroids make it very difficult to establish evolutionary trends and exact relations of these orders. The Neuroptera (in the broad sense) and Mecoptera are the most primitive orders and probably originated from a common stock. Although Neuroptera and Mecoptera are closely related they are placed here as representing two lines of evolution: The Panneuroptera including Neuroptera, Megaloptera, Raphidiodea, Coleoptera, Strepsiptera and Hymenoptera, and the Panmecoptera (= Panorpoidea) including Mecoptera, Trichoptera, Lepidoptera, Diptera and Siphonaptera. In both groups there is a tendency toward developing sucking mouth-parts. At the base of the two groups (Mecoptera-Neuroptera) we find that the mesothoracic coxa is divided into eucoxa and meron, but in the Panneuroptera line this division of the coxa is lost (Coleoptera, Strepsiptera, and Hymenoptera). On the other hand, most of the forms of the Panmecoptera retain the meron. Males of the Panneuroptera tend to develop processes on the gonocoxopodites, usually called volsellae (found in Neuroptera, Cupedidae in Coleoptera, and Hymenoptera).

Recently, HINTON (17) has elevated the family Boreidae of Mecoptera to ordinal rank (Neomecoptera). The main distinctions between the Boreidae and Mecoptera proper are given by Hinton as follows:

LARVA:

1) The cranium is without a distinct epistomal suture.
2) The cardo is not fused to the basistipes, and the tentorial adductors of the cardo are present; if homologues of these muscles are present in the Mecoptera they are now tentorial adductors of the cardostipes.
3) The postmentum is well developed instead of lost or reduced to an articulating membrane between the prementum and the cranium.
4) The abdomen lacks prolegs, whereas in the Mecoptera prolegs are present on the first eight abdominal segments.
5) The larvae feed on moss, whereas those of the Mecoptera are carnivorous.

ADULT:

1) The ovaries are panoistic instead of polytrophic as in the Mecoptera.
2) The 11th abdominal segment of the female lacks cerci, whereas one-or two-segmented cerci are present in the Mecoptera.
3) The ninth and tenth abdominal segments are modified to form a large functional ovipositor but no such "ovipositor" is found in the Mecoptera.
4) The adult gut lacks the six special rectal glands of the Mecoptera.

Although the Boreidae seem actually a well differentiated group among the Mecoptera, it still has a great number of characters in common with that order, indicating its relation with it. For this reason, it would be more logical to retain the Boreidae as a suborder of Mecoptera, rather than forming an entirely new order for it.

The relation of Trichoptera to Mecoptera is well illustrated by comparing the wing venation of a primitive Caddis-fly such as *Stenopsychodes hiemalis* (Policentroprodidae) with that of the fossil group Paratrichoptera (a mecopteron). They resemble each other as follows: A four-branched Rs; stalk of $R_2 + _3$ longer than that of $R_4 + _5$; a four-branched M; stalk of $M_1 + _2$ longer than that of $M_3 + _4$; with a closed radial cell (re), and a closed median cell (mc). The Lepidoptera are very closely related to Trichoptera (Table 6); it seems that both originated from a common ancestor. The Micropterygidae, although the most primitive of all the Lepidoptera, have been usually regarded as belonging to that order. However, HINTON (16) has suggested that it should be regarded as a distinct order (Zeugloptera). Although there is no reason for excluding the Micropterygidae from the Lepidoptera on the basis of adult and pupa structure, HINTON (17) in a recent paper listed the following important differences between the larva of the Micropterygidae and Lepidoptera:

1) The cranium of the Zeugloptera lacks the adfrontal ridge and adfrontal sutures.
2) The tentorial bridge is short and broad as in the Mecoptera instead of being long and narrow.
3) The anterior tentorial pit is close to the inner side of the antennal base instead of far behind the antenna.
4) The maxilla has a separate galea and lacinia instead of an undivided lobe.
5) A cranial flexor of the dististipes is present.
6) Lateral labral retractors are present.
7) A pair of cibarial muscles is inserted in the labrum.

8) A spinneret is absent.
9) The leg has the coxa, trochanter, and femur fused but the tarsus and pretarsus are discrete segments, a type of reduction unknown in the Lepidoptera.
10) The ventral abdominal prolegs lack retractor muscles.
11) The spiracles of the metathorax are functional instead of non-functional.
12) The chaetotaxy of the thorax and abdomen is of quite a different type the distribution of the primary setae of the two orders is entirely different.

Furthermore, he adds that "in many respects, especially in the structure and musculature of the head and mouth-parts of the larva, the Zeugloptera appear to be much more closely related to the Trichoptera than to the Lepidoptera."

TABLE 6

Similarities between Trichoptera and Lepidoptera

Trichoptera	Lepidoptera
Mandibles atrophied or vestigial in many genera	In the majority the mandibles are wanting (present and functional in Micropterygidae)
In the genus *Dipsendopsis* each maxillary lobe is in the form of a pendulous or an annulated half-tube	The annulated half-tube elongation of each maxillary lobe of *Dipsendopsis* recalls the condition found in certain archaic Lepidoptera in which the two elements of the proboscis are not coadapted
Certain Trichoptera (*Platrotarsus*) have scattered scales on the wings; however, they are narrow and acuminate, with few striae	Wings clothed with scales
The wing venation of *Rhyacophila* is very generalized. Almost all the veins are longitudinal, not more than two veinlets in the costal series are retained, and the cross-veins are reduced in number	The wing venation of *Rhyacophila* closely resembles that of the most primitive Lepidoptera

The Diptera seem to have originated from a Mecoptera-like insect. This is indicated by the following features in common:

1) The protodipteron, *Permotipula patricia* (a tipuloid dipteron) has a wing venation very similar to that of Paratrichoptera, the main difference between the two is the narrowing of the wing base in *Permotipula*, and as a consequence the third anal vein is missing, and the second one reduced.
2) According to TILLYARD (41) the family Nannochoristidae (Mecoptera) are the closest living relatives of the Diptera. This family has a head-capsule and mouth-

parts similar to that of some flies. This is well seen by comparing the head of *Nannochorista* with that of *Edwardsina* (Blepharoceridae). In both types we find five-segmented maxillary palpi, with a very short basal segment and the third with a peculiar sense-organ; two-segmented labial palpi, which are placed very close together forming a labellum-like structure. It should be pointed out that CRAMPTON (4, 5), has indicated that the labellar lobes are probably the labial palpi in Diptera. Also the labium-epipharynx and mandibles are elongated (bur more so in *Edwardsina*).

3) The genitalia of certain Diptera are very similar to those of Mecoptera. For example, in the Tanyderidae (Diptera) and in the Meropeidae (Mecoptera) the gonopods are transformed into large forceps with reduction of all the other parts.

As to the Siphonaptera the origin is still more doubtful, perhaps both Diptera and Siphonaptera originated from a common ancestor in the Mecoptera-Trichoptera complex.

With the orders of Panneuroptera one is faced immediately with unsolved phylogenetic problems, and all that can be done here is merely to mention some of the suggestions which have been made.

The similarity of certain coleopterous larvae with those of Neuroptera may indicate a possible relationship. The family Cupedidae, considered to be the most primitive of all the Coleoptera, shows reticulated elytra, simulating veins. This, it is said, may indicate that the elytra of Coleoptera may have arisen by a condensation of the veins from a wing like that of Neuroptera. In a very recent paper, CROWSON (7) has pointed out that "the remarkable lower Permian fossil *Tshekardocoleus* looks like an intermediate form between cupedid-type Coleoptera and Megaloptera of the *Corydalis* type and may be taken as direct evidence in support of the neuropteran theory... The fossil evidence seems to be at least consistent with the theory that Coleoptera took their origin from megaloptera-like ancestors during the Permian period, very soon after the first adaptive radiation of the Endopterygota."

That Strepsiptera are related to Coleoptera is and has been the general opinion among entomologists. But JEANNEL (19) has pointed out that the Strepsiptera seem to be more closely related to Hymenoptera than to Coleoptera. This is supported, according to him, by the following characters shared by both the Strepsiptera and Hymenoptera.

1) First abdominal segment incorporated to the thorax (Clistogastra among the Hymenoptera), and with epimeron and episternum obliquely placed.
2) The head completely free from the prothorax, and orthognathous* (this is different from that of most Coleoptera).
3) The flabellated antennae of Strepsiptera are as similar to those of some tenthredinids as to those of *Rhipidius*.
4) The legs of Mengeidae are of the hymenopterous type. A five-segmented tarsus with the basal segment (basitarsus) enlarged. The structure of the empodium resembles that of the Hymenoptera.
5) The triungulins of Strepsiptera are actually more similar to the planidium of chalcids than to those of meloids and rhipiphorids.

---

* This condition is called "hypognathous" by American authors.

6) The Strepsiptera and the Hymenoptera are the only two orders of pterygotes in which the embryo has only one envelope instead of the usual two (amnion and serosa).
7) Polyembryony is known to occur in Halictophagidae, and also in some Hymenoptera, especially in chalcids.

However, it should be pointed out that most of the above characters are also found in some Coleoptera and furthermore many of these characters can be explained as due to other reasons than relationship. Thus, the doubled antennae of Strepsiptera probably arose quite independently from similar conditions in either Hymonoptera of Coleoptera. Although the Strepsiptera and Hymenoptera have only one envelope in the developing embryo, it is the amnion which is present in the Strepsiptera, while in Hymenoptera it is the serosa. Actually, it appears, as CROWSON (7) has indicated, "that no features of the Stylopoidea are inconsistent with a derivation of the group from normal coleopterous ancestors." He gives the following features as an indication of the relationship of the Strepsiptera and Coleoptera:

1) The use only of the hind wings in flight.
2) The more extensive sclerotization of the abdominal sternites than of the tergites (contrary to the usual endopterygote condition).
3) The nature of the metendosternite.
4) The structure of the first-instar larva, which does not seem to differ from similar "triungulins" of Meloidae and Rhipiphoridae in any character which could possibly be regarded as of ordinal importance.

CONCLUSIONS

1) Our present knowledge seems to indicate that the closest relatives of insects are the myriapods, of which, the Symphyla have the greatest number of characters in common with most primitive insects. This may suggest that the Insecta and Symphyla were derived from a common stock of myriapod-like arthropods. There is also some evidence that the insects may have originated by neoteny from myriapod-like arthopods.

2) The exclusion of the entognathous apterygotes from the class Insecta does not seem to be well justified.

3) There is good evidence that the entognathous apterygotes (Protura, Collembola, Diplura) are interrelated.

4) Since the differences between lepismatids and machilids include several features of great phylogenetic importance (Table 1), they are regarded here as representing two orders: the Microcoryphia, including the machilids, and the Thysanura proper, including the lepismatids.

5) The origin of the pterygotes from the apterygotes is still a question of much speculation. There are three main theories as to the origin of the wings; all these views agree in that the wings were developed from paranotal processes on the meso- and meta-thorax. a) According to MARTYNOV the paranotal extensions tended to grow sidewards and were used as gliding organs.

The wings originated by the acquisition of a line of flexibility at the base of each process. Later some acquired the ability to flex the wings over the back of the abdomen. b) SCHWANWITSCH, on the other hand, believes that the paranota were directed backwards and became movable from the very beginning of their evolution. c) Finally, according to LEMCHE, wings originated from two different types of growth of the paranotal processes. In one, the growth was sidewards, giving rise to the Palaeodictyoptera, Protohemiptera, Protodonata, Odonata, and Megasecoptera. In the other, growth was backwards and gave origin to the rest of the orders.

6) Of all the living groups of Pterygota the Ephemeroptera seem to be the most primitive. The Odonata are an isolated group, this is also true of the Ephemeroptera. However, of all the living orders of Pterygota the Ephemeroptera are the closest to Odonata.

7) The Neopteran orders comprise three main evolutionary trends or groups: The orthopteroid, hemipteroid, and neuropteroid lines (Table 2).

8) In the orthopteroid group two lines can be distinguished: The orthopteroid proper, with clear affinities, and a line (Panplecoptera) represented by Plecoptera, Embioptera, and Dermaptera (Table 3) with doubtful affinities. In the orthopteroid proper two other lines can be recognized: The Panorthoptera, represented by Grylloblattodea, Saltatoria, and Phasmida; the Dictyoptera, represented by Blattaria, Mantodea and Isoptera (Table 4). Although the termites superficially seem to be far apart from the roaches, careful examination will reveal a close relation of these two groups (Table 5).

9) The main evolutionary trend in the hemipteroids is a gradual development of sucking mouth-parts.

10) Two lines of evolution can be recognized in the neuropteroid group: The Panneuroptera including Neuroptera, Megaloptera, Raphidiodea, Coleoptera, Strepsiptera and Hymenoptera, and the Panmecoptera (= Panorpoidea) including Mecoptera, Trichoptera, Lepidoptera, Diptera and Siphonaptera. In both groups there is a tendency toward developing sucking mouth-parts.

By necessity, the above account has been somewhat superficial and to a certain degree dogmatic. This, it is hoped, shall be corrected in the future as our knowledge on these matters expands. It is also hoped that more and more entomologists shall become aware of the scientific importance of this fascinating and everlasting challenge which the phylogeny and the interrelationship of the insect orders offer to anyone interested.

RESUMEN

El trabajo presente consiste en un análisis y evaluación de la literatura sobre la evolución e interrelación de los órdenes de los insectos, dando como resultado las siguientes conclusiones:

1) Nuestros conocimientos actuales parecen indicar que los artrópodos más cercanos a los insectos son los miriápodos, de los cuales los Symphyla poseen el mayor número de caracteres en común con los insectos primitivos. Esto

parece sugerir que Insecta y Symphyla se derivaron de un tronco común, formado de artrópodos de tipo miriápodo. Hay evidencia también de que los insectos pueden haberse originado por medio de neotenia de dicho tronco común.

2) La tendencia reciente de excluir los apterigotos entognatos de la clase Insecta no parece estar bien justificada.

3) Investigaciones recientes indican que los apterigotos entognatos (Protura, Collembola y Diplura) están interrelacionados.

4) En vista de que las diferencias entre lepismátidos y maquílidos incluyen varias características de gran importancia filogenética (Cuadro 1), se considera que forman dos órdenes: Microcoryphia, que comprende a los maquílidos, y Thysanura propiamente dichos, o sea los lepismátidos.

5) El origen de los pterigotos aun continúa sin solución.

6) De todos los grupos vivientes de Pterygota los Ephemeroptera parecen ser los más primitivos. Los Odonata forman un grupo aislado, lo cual también es cierto de los Ephemeroptera, pero de todos los órdenes vivientes de Pterygota los efemerópteros son los que más se aproximan a los odonatos.

7) Los órdenes neópteros comprenden tres grupos o tendencias evolucionarias principales: Las líneas Orthopteroidea, Hemipteroidea y Neuropteroidea (Cuadro 2).

8) En el grupo ortopteroideo se pueden distinguir dos líneas: los Orthopteroidea propiamente dichos, con afinidades claras, y una línea (Panplecoptera) representada por Plecoptera, Embioptera, y Dermaptera (Cuadro 3), con afinidades dudosas. En el grupo ortopteroideo propiamente dicho pueden reconocerse otras dos líneas: los Panorthoptera, representados por Grylloblattodea, Saltatoria, y Phasmida; los Dictyoptera, representados por Blattaria, Mantodea e Isoptera (Cuadro 4). Aunque los comejenes (Isoptera) superficialmente parecen estar muy lejanos de las cucarachas, un examen cuidadoso revelará una relación cercana entre los dos grupos (Cuadro 5).

9) La principal tendencia evolucionaria en los hemipteroideos es un desarrollo gradual de un aparato bucal chupador.

10) Dos líneas de evolución pueden ser reconocidas en el grupo neuropteroideo: los Panneuroptera incluyendo Neuroptera, Megaloptera, Raphidiodea, Coleoptera, Strepsiptera e Hymenoptera, y los Panmecoptera (= Panorpoidea) incluyendo Mecoptera, Trichoptera, Lepidoptera, Diptera y Siphonaptera. En ambos grupos hay una tendencia hacia el desarrollo de un aparato bucal chupador.

SCHLUSSFOLGERUNGEN

Ein ausführliches Studium und Schätzung der Literatur über die Evolution und Beziehungen der verschiedenen Insektenordnungen erlauben dem Verfasser wie folgt abzuschliessen:

1) Die gegenwärtige Kenntnis scheint hinzuweisen, dass die Myriapoden die den Insekten nähre Gliederfüssler sind, von denen die Symphila die meisten charakteristischen Merkmale gemeinsam mit den ursprünglichen Insekten besitzen. Das lässt schliessen, dass Insecta und Symphila von einem gemeinsamen, von

myriapoden-artigen Gliederfüsslern gebildeten Stamm sich ableiten. Es besteht auch Merkmale, die darauf hinweisen, dass die Insekten vermittels Neotenie von besagten Stamm herrühren.

2) Es scheint, dass es keinen Beweis gibt, um die entognatischen Apterygoten von der Klasse Insecta auszuschliessen.

3) Neueste Untersuchungen weisen darauf hin, dass die entognatischen Apterygoten (Protura, Collembola, Diplura) in Beziehung zueinander sind.

4) Da die Unterschiede zwischen Lepismatiden und Machiliden mehrere Merkmale von grosser phylogenetischer Bedeutung einschliessen (Tafel 1), betrachtet der Verfasser die Bildung zweier Ordnungen: die Microcoryphia, welche die Machiliden umfassen, und die Thysanura, welche die Lepismatiden sind.

5) Der Ursprung der Pterygoten bleibt nach wie vor im Ungewissen.

6) Von allen lebenden Gruppen der Pterygoten scheinen die Ephemeroptera die Primitivsten zu sein. Die Odonata bilden eine Gruppe für sich, was auch für die Ephemeroptera erwiesen ist, aber von allen lebenden Ordnungen der Pterygota sind es die Ephemeroptera die am meisten den Odonata ähnlich sind.

7) Die Ordnungen der Neoptera bestehen aus drei Gruppen oder hauptsächlichen evolutionären Bestrebungen: die Orthopteroidea; Hemipteroidea und Neuropteroidea (Tafel 2).

8) In der Gruppe der Orthopteroidea kann man zwei Linien unterscheiden: die tatsächlichen Orthopteroidea mit bestimmten Affinitäten und eine Linie (Panplecoptera) gebildet durch Plecoptera, Embioptera und Dermaptera (Tafel 3) mit undeutlichen Affinitäten. In der Gruppe der tatsächlichen Orthopteroidea lassen sich weitere zwei Linien bestimmen: die Panorthoptera, gebildet durch Grylloblattoidea, Saltatoria und Phasmida; die Dictyoptera, gebildet durch Blattaria, Mantodea und Isoptera (Tafel 4). Wenngleich die Termiten oberflächlicherweise weit entfernt von den Schaben sind, ein genaues Examen wird eine nahe Beziehung zwischen beiden Gruppen aufweisen (Tafel 5).

9) Die vorherrschende evolutionäre Bestrebung bei den Hemipteroidea ist eine abgestufte Entwicklung des Saugwerkzeuges.

10) Zwei evolutionäre Linien lassen sich in der Gruppe der Neuropteroidea erkennen: die Panneuroptera einschliesslich die Neuroptera, Megaloptera, Raphidiodea, Coleoptera, Strepsiptera und Hymenoptera, und die Panmecoptera (= Panorpoidea) einschliesslich die Mecoptera, Trichoptera, Lepidoptera, Diptera und Siphonaptera. In beiden Gruppen besteht die Tendenz zur Entwicklung eines Saugapparates.

LITERATURE CITED

1. Beer, G. R. de
1940. *Embryos and ancestors.* x + 108 pp., Oxford University Press.

2. Carpenter, F. M.
1953. The evolution of Insects. *American Scientist,* 41: 256-270.

3. COMSTOCK, J. H.
1918. *The wings of Insects*. 430 pp., 10 pls., Ithaca, Comstock Publishing Co.

4. CRAMPTON, G. C.
1923. A comparison of the labium in certain holometabolous insects from the standpoint of phylogeny. *Proc. Ent. Soc. Wash.*, 25: 171-180.

5. CRAMPTON, G. C.
1925. A phylogenetic study of the labium of holometabolous insects, with particular reference to the Diptera. *Proc. Ent. Soc. Wash.*, 27: 68-91.

6. CRAMPTON, G. C.
1938. The interrelationships and lines of descent of living insects. *Pomona Jour. Ent. Zool.*, 45: 165-181.

7. CROWSON, R. A.
1960. The phylogeny of Coleoptera. *Ann. Rev. Ent.*, 5: 111-134.

8. DELAMARE-DEBOUTEVILLE, C.
1948. Sur la morphologie des adultes aptères et ailés de Zoraptères. *Ann. Sci. Nat.*, 9: 145-154.

9. DELAMARE-DEBOUTEVILLE, C.
1948. Observations sur l'écologie et léthologie des Zoraptères. La question de leur vie sociale et de leurs prétendus rapports avec les termites. *Rev. ent.*, 19: 347-352.

10. EDMUNDS, G. F. JR., & J. R. TRAVER
1954. The flight mechanics and evolution of the wings of the Ephemeroptera, with notes on the archetype insect wing. *Jour. Wash. Acad. Sci.*, 44: 390-400.

11. EWING, H. E.
1943. The origin and classification of the Apterygota. *Proc. Ent. Soc. Wash.*, 44: 75-98.

12. FOLSOM, J. W.
1900. The development of the mouth-parts of *Anurida maritima*. *Guér. Bull. Mus. Comp. Zool.*, 36: 87-157.

13. HANSEN, H. J.
1930. *Studies on Arthropoda III. On the comparative morphology of the appendages in the Arthropoda*. 376 pp., 16 pls. Copenhagen.

14. HENNING, W.
1953. Kritische Bemerkungen zum phylogenetischen System der Insekten. *Beitr. Ent.*, 3: 1-85.

15. HENRIKSEN, K.
1932. The manner of moulting in Arthropods. *Notul. Ent.*, 11: 103-127.

16. HINTON, H. E.
1946. On the homology and nomenclature of the setae of Lepidopterous larvae, with some notes on the phylogeny of the Lepidoptera. *Trans. R. Ent. Soc. Lond.*, 97: 1-37.

17. HINTON, H. E.
1958. The phylogeny of the panorpoid orders. *Ann. Rev. Ent.*, 3: 181-206.

18. IMMS, A. D.
1945. The Phylogeny of Insects. *Tijdschr. Ent.*, 88: 63-66.

19. JEANNEL, R.
1945. Sur la position systémtique des Strepsiptères. *Rev. Franc. Ent.*, 11: 11-118.

20. JOHANNSEN, O. A. & F. H. BUTT
1941. *Embryology of insects and myriapods.* xi + 462 pp., 370 figs. McGraw-Hill Book Company, Inc.

21. JUDD, W. W.
1948. A comparative study of the proventriculus of Orthopteroid insects with references to its use in taxonomy. *Canad. J. Res.* (D), 26: 93-161.

22. LEMCHE, H.
1940. The origin of winged insects. *Vidensk. Medd. Dansk Naturh. Foren.*, 104: 127-168.

23. MANTON, S. M.
1953. Locomotory habits and the evolution of the larger arthropodan groups. *Symp. Soc. exp. Biol.*, 7: 339-476.

24. MARCUS, E.
1958. Evolution of the animal phyla. *Quart. Rev. Biol.*, 33: 24-58.

25. MARTYNOV, A. V.
1925. Über zwei Grundtypen der Flügel bei den Insekten und ihre Evolution. *Zeit. Morph. Oekol. Tiere*, 4: 465-501.

26. MATSUDA, R.
1956. Morphology of the thoracic exoskeleton and musculature of a mayfly *Siphlonurus columbianus* McDunnough (Sipholonuridae, Ephemeroptera). *Jour. Kansas Ent. Soc.*, 29: 92-113.

27. REMINGTON, C. L.
1954. The suprageneric classification of the order Thysanura. *Ann. Ent. Soc. Amer.*, 47: 277-286.

28. REYNE, A.
1927. Untersuchungen über die Mundteile der Thysanopteran. *Zool. Jahrb., Anat.*, 49: 391-500.

29. ROSS, H. H.
1955. Evolution of insect orders. *Ent. News*, 66: 197-208.

30. SCHWANWITSCH, B. N.
1943. Subdivision of Insecta Pterygota into subordinate groups. *Nature*, London, 152: 727-728.

31. SCHWANWITSCH, B. N.
1946. On the interrelations of the orders of Insecta Pterygota as dependent on the origin of flight. *Zool. Journ.* (Moscow) 24: 529-542.

32. SIMPSON, G. G.
1945. The principles of classification and a classification of mammals. *Bull. Amer. Mus. Nat. Hist.*, 85:1-350.

33. SNODGRASS, R. E.
1935. *Principles of insect morphology.* ix + 667 pp., 319 figs. Mc-Graw-Hill Book Company, Inc., New York.

34. SNODGRASS, R. E.
1944. The feeding apparatus of biting and sucking insects affecting man and animals. *Smithsonian Misc. Coll.*, 104: 1-113.

35. SNODGRASS, R. E.
1951. *Comparative studies on the head of mandibulates arthropods.* viii + 118 pp., 37 figs. Comstock Publishing Company, Inc.

36. SNODGRASS, R. E.
1958. Evolution of arthropod mechanisms. *Smithsonian Misc. Coll.*, 138: 1-77.

37. TIEGS, O. W.
1940. The embryology and affinities of the Symphyla based on a study of *Hanseniella agilis*. *Quart. Journ. Micr. Sci.*, 82: 1-225.

38. TIEGS, O. W.
1945. The post-embryonic development of *Hanseniella agilis* (Symphyla). *Quart. Journ. Micr. Sci.* 85: 191-328.

39. TIEGS, O. W. & S. M. MANTON
1958. The evolution of the Arthropoda. *Biol. Rev. Cambridge Phil. Soc.* 33: 255-337.

40. TILLYARD, R. J.
1932. Kansas Permian insects. Pt. 15. The order Plectoptera. *Amer. Jour. Sci.*, 23: 95-134. 237-272.

41. TILLYARD, R. J.
1935. The evolution of the scorpion flies and their derivatives. *Ann. Ent. Soc. Amer.*, 28: 1-45.

42. TUXEN, S. L.
1931. Monographie der Proturen. I. Morphologie nebst Bemerkungen über Systematik und Oekologie. *Z. Morph. Oekol. Tiere*, 22: 671-720. 20 figs.

43. TUXEN, S. L.
1959. The phylogenetic significance of entognathy in entognathous apterygotes. *Smithsonian Misc. Coll.*, 137: 379-416.

44. WEBER, H.
1936. Copeognatha. *Biologie der Tiere Deutschlands*. 39^{27}: 1-50.

45. WEBER, H.
1938. Beiträge zur Kenntnis der Überordnung Psocoidea. I. Die Labialdrüsen der Copeognathen. Zool. Jahrb., Anat., 64: 243-286.

46. WEBER, H.
1954. *Grundriss der Insektenkunde*. xi - 428 pp., 220 figs. Gustav Fisher Verlag Stuttgart, 3rd. Edn.

Rev. Biol. Trop., 8 (1): 123-143, 1960

Inoculación experimental de Sporotrichum schenckii en embrión de pollo*

por

German F. Sáenz**

(Recibido para su publicación el 21 de abril de 1960)

En 1941, MOORE (8) inoculó el *Sporotrichum schenckii* en membrana coriolantoica de embriones de pollo, abarcando su estudio, de un modo preferente, el aspecto histopatológico de las lesiones encontradas. En 1951, BRUECK y BUDDINGH (1) utilizando la vía del saco vitelino, inocularon pus de lesiones humanas, con miras a demostrar el valor de este método para el aislamiento y diagnóstico de la infección.

No existiendo en la literatura otros trabajos referentes a este tema, se inició un estudio en el cual se deseaba conocer las variaciones morfológicas que podría sufrir especialmente la fase micelial del hongo, al ser inoculada por diferentes vías en huevos en diversos estadios de desarrollo. Además se pretendió comprobar si habría diseminación del hongo a otras regiones embrionarias a partir de la inoculación en una área determinada.

En el presente trabajo se relatan los resultados obtenidos en las inoculaciones experimentales de *S. schenckii* en huevos embrionados por diez vías diferentes, indicándose también, aunque en forma somera, las modificaciones anatómicas e histológicas de las regiones embrionarias afectadas.

MATERIAL Y METODOS

Se utilizaron huevos embrionados, de gallinas Leghorn, de un peso promedio de 60 gramos. Usamos en la mayoría de los casos lotes de cinco para cada tipo de inoculación, los cuales recibieron de 0.1 a 0.3 ml de suspensión

---

* Trabajo basado en la Tesis de Grado del mismo nombre, presentada a la Facultad de Microbiología de la Universidad de Costa Rica.

** Departamento de Parasitología, Escuela de Microbiología, Universidad de Costa Rica.

del hongo. Asimismo, dos o tres huevos fueron inoculados con solución salina y usados como testigos. Los embriones permanecieron siempre a 37.5°C y a una humedad relativa de 50 por ciento. Las inoculaciones se efectuaron en períodos comprendidos entre los 4 y 18 días.

Para la obtención de la fase micelial del hongo se utilizó una cepa de *S. schenckii* (fig. 1) recién aislada de un caso gomoso humano. El hongo fue cultivado a temperatura ambiente (aproximadamente 22°C) por 72 a 96 horas, en 5 ml de Sabouraud glucosado. Antes de efectuar las inoculaciones, se centrifugaron los tubos, suspendiendo el sedimento en un volumen aproximado de 4 ml de solución salina estéril.

Fueron realizados además, unos pocos experimentos usando la fase levaduriforme del hongo (fig. 2), obtenida en cultivos de agar-sangre con 2 por ciento de glucosa a 37°C.

En general se adoptaron las consideraciones técnicas clásicas relativas a inoculaciones en embriones de pollo (2). Las pequeñas modificaciones que se introdujeron a las técnicas se refieren en especial a la apertura inicial de los huevos.

Los embriones fueron inoculados del siguiente modo:

Saco vitelino	7 días
Membrana corioalantoica	11 días
Cavidad alantoica	11 días
Saco del albumen	7 días
Cavidad amniótica	11 días
Intracerebral	10-18 días
Intramuscular	13-16 días
Endovenosa	13-16 días
Membrana de la cáscara	4-15 días

El material sospechoso obtenido en los huevos inoculados se sembró de rutina en Sabouraud líquido glucosado, en el agar glucosado de Sabouraud y, en agar sangre, para comprobar principalmente la ausencia de bacterias.

En cada uno de los embriones se hizo un estudio macro y microscópico de la región inoculada y de los anexos, líquidos y órganos embrionarios. Los frotes fueron teñidos por los métodos de Gram (5) y del ácido peryódico-Schiff (técnica P.A.S.) (7). Piezas de anexos y órganos del embrión se fijaron en formalina al 10 por ciento, se incluyeron en parafina y los cortes obtenidos se tiñeron por los métodos de hematoxilina-eosina (6), del P.A.S. y del Gram para tejidos (5).

RESULTADOS

Inoculación en saco vitelino

Cinco embriones murieron a los 3, 4, 9, 11 y 13 días después de inoculados, no mostrando mayores alteraciones a no ser en los últimos un moderado subdesarrollo.

El anexo vitelino presentó en general, un mayor tamaño y un engrosamiento de su pared. En los dos últimos embriones no se observó indicio alguno que indicara el proceso de invaginación del saco vitelino a la cavidad abdominal. En los restantes anexos embrionarios no notamos alteraciones ostensibles.

Respecto a las características del hongo, la predominancia de formas vegetativas micelianas iniciales, fueron sustituídas finalmente por numerosas formas blastospóricas y en seudomicelio. Las formas en cigarro se hallaron en todos los casos, con mayor frecuencia en los últimos embriones (figs. 3, 4). Pudimos notar también, que el crecimiento del hongo era más acentuado en el área subyacente al saco vitelino (fig. 5), nunca intracelularmente y, que la infección no se extendió a otros terrenos embrionarios.

Testigos: nacen a los 22 días.

Inoculación en membrana corioalantoica (M.C.A.)

Cinco embriones murieron entre el cuarto y sétimo días después de inoculados sin mostrar anormalidades en cuanto al tamaño, pero sí manifestaciones hemorrágicas. Macroscópicamente las membranas corioalantoicas mostraron regiones edematosas y pálidas, especialmente en el área de inoculación. Microscópicamente las lesiones eran de tipo granulomatoso con predominio de glóbulos rojos y monolinfoides. En los otros anexos no se notaron anormalidades evidentes.

En los embriones que murieron al sétimo día se observó la predominancia de la fase parasitaria o tisular del hongo, con las típicas formas de cigarro, notándose además un paso progresivo bien definido a partir del inóculo micelial, encontrándose como elementos intermediarios, levaduras mono y multigemantes, seudomicelios cortos y pleomórficos, formas redondeadas y escaso promicelio (figs. 6, 7, 8).

La inoculación en la M.C.A. provoca un crecimiento localizado del hongo con escasa tendencia a difundirse a otros anexos o al propio embrión. En los cortes histológicos se pudo demostrar un mayor crecimiento del hongo en la región subcoriónica (fig. 9).

Testigos: nacen a los 22 días.

Inoculación en cavidad alantoica

Un embrión murió a los tres días y otro a los nueve. Tres más fueron matados intencionalmente a los 4, 5 y 7 días después de inoculados con el propósito de estudiar en forma progresiva tanto el líquido alantoico como las características del hongo.

Conforme avanzaba el período de incubación, se pudo notar el predominio manifiesto de la fase levaduriforme (fig. 10). A partir de los últimos embriones los cultivos resultaron ser positivos pero en forma escasa y, los frotes mostraron pocos elementos del hongo. En general, no logramos observar

anormalidades claras en los embriones o anexos, a no ser los alantoicos moderadamente opacos y el saco vitelino engrosado en el último embrión. El líquido alantoico al principio grumoso y de color amarillo claro, tornóse al final blanquecino y casi sin grumos.

En los cortes efectuados se pudo demostrar que los anexos alantoicos no mostraban una reacción celular manifiesta; asimismo, se comprobó la escasez de los elementos del hongo, hecho que corrobora los resultados obtenidos en el estudio de los frotes y de los cultivos.

Testigos: nacen a los 22 días.

Inoculación en cavidad extraembrionaria

Cinco embriones murieron en un período comprendido entre el cuarto y sexto días después de inoculados, mostrando alteraciones en su aspecto y tamaño, viéndose pálidos y subdesarrollados.

De un modo general, todos los anexos embrionarios mostraron anormalidades evidentes, observándose exentos de sangre y fláccidos. El líquido amniótico en la mayoría de los casos se presentó hemorrágico; el extraembrionario en los últimos embriones tornóse mucoide y escaso, bañando a manera de un exudado, los anexos circundantes de la cavidad extraembrionaria.

Las características del hongo tanto en el líquido extraembrionario como en los anexos contiguos, nos demuestran las propiedades pleomórficas del mismo. En los primeros huevos, este pleomorfismo se hace exagerado, para luego encontrar en los embriones que murieron posteriormente, un predominio de las formas seudomicelianas y de micelio vegetativo (figs. 11, 12, 13).

Testigos: nacen a los 23 días.

Inoculación en saco del albumen

De un lote de cinco huevos inoculados, se abrieron dos de ellos al octavo y noveno días con el propósito de comprobar la posible diseminación del hongo a la cavidad amniótica. Sin embargo, tanto en éstos como en un tercero que murió a los 14 días después de inoculado, no se logró demostrar tal proceso. Los dos restantes del lote nacieron normalmente. Los embriones estudiados no mostraron anormalidades ostensibles y el albumen en general presentó modificaciones ligeras en su aspecto, cantidad y consistencia.

El albumen resulta ser un medio desfavorable para el hongo dada la pobreza de los cultivos positivos y por la escasez de los elementos del hongo en los frotes estudiados.

Desde el punto de vista morfológico se encontraron inicialmente formas micelinas irregulares débilmente coloreadas, blastosporas alargadas generalmente agemantes y, un seudomicelio pleomórfico (fig. 14). Del embrión que murió obtuvimos algún material albuminoideo de la región contigua a los tejidos propios del saco del albumen, logrando constatar que poseía principalmente elementos levaduriformes y en cigarro (fig. 15). También encontramos positivo el anexo vitelino en este caso. En un resto albuminoideo de uno de los

embriones nacidos, observamos elementos cocoides débilmente teñidos, algunos con cortas proyecciones, otros con su pared rota y otros en desintegración (fig. 16).

Testigos: nacen a los 22 días.

Inoculación en cavidad amniótica

Cinco embriones murieron en un período comprendido entre el primero y segundo días después de inoculados y presentaron principalmente alteraciones en su aspecto, viéndose pálidos con zonas definidas de hemorragia. Por lo común todas las membranas extraembrionarias presentaron modificaciones en su aspecto y consistencia, siendo la más afectada la amniótica. El líquido amniótico siempre se mostró hemorrágico.

Debido probablemente al pequeño período de incubación post-inoculatorio, no logramos demostrar una diseminación del hongo a los tejidos intrínsecos del embrión, aunque sí escasamente a otros anexos y al líquido alantoico en los dos últimos embriones.

En el material amniótico (membrana y líquido), las características del hongo variaron desde las formas micelianas vegetativas, predominantes en los primeros casos (fig. 17), hasta la aparición de elementos principalmente redondeados y en seudomicelio (fig. 18). También se observaron en todos los frotes, conidias pequeñas, promicelio y células multigemantes (fig. 19). En las preparaciones efectuadas a partir de líquido traqueal logramos ver escasos elementos primordialmente redondeados pero no típicamente levaduriformes.

Testigos: de seis testigos, uno murió a las 40 y otro a las 72 horas; dos a los 5 y 6 días respectivamente y, dos nacieron a los 23 días.

Inoculación intracerebral

La inoculación intracerebral del *S. schenckii* siempre fue fatal. En general notamos que embriones de 10 a 14 días son más susceptibles muriendo entre las 6 y 16 horas, mientras que los de 16 a 18 días, resistieron un mayor lapso de tiempo post-inoculatorio (hasta 32 horas). Todos los embriones presentaron un aspecto amarillento, con hemorragia generalizada en toda la bóveda craneana, inclusive en los ojos. Los anexos embrionarios denotaban alteraciones en su aspecto e irrigación.

La inoculación de la fase levaduriforme prolonga un poco más el período de supervivencia en embriones de 16 días. De cinco de ellos, uno murió a las 80 horas y los cuatro restantes entre las 48 y 70 horas después de la inoculación.

En el estudio de las preparaciones efectuadas a partir de material cerebral, en la primera experiencia, notamos la presencia de micelio profuso muy ramificado, con excepcionales conidias laterales. No encontramos blastogénesis. La mayor parte del micelio dio una débil reacción P.A.S. positiva.

La reacción celular encontrada en los cortes de cerebro fue localizada

y especialmente encontramos elementos linfoides, plasmacelulares y células rojas. En los mismos cortes se lograron observar "nidos micelianos", rodeados de una zona de necrosis manifiesta. En la inoculación de blastosporas, también se observaron "nidos levaduriformes" y necrosis moderada (fig. 20).

De seis testigos con 17 días, cuatro murieron a las 24 horas y dos a las 80 horas siguientes a la inoculación.

Inoculación intramuscular

Al inocular embriones de pollo por la vía intramuscular, se lograron diversos resultados según la edad de los embriones utilizados. De un lote de cinco embriones de 12 días, dos de ellos sobrevivieron, uno de los cuales presentó anormalidades anatómicas en la pata comprometida. Ninguno evidenció proceso infeccioso alguno. De los fallecidos uno murió a las 24 horas y dos a las 72 horas después de la inoculación. Todos ellos mostraron hemorragia y edema generalizado en todo el miembro.

En general, tanto en los embriones como en los anexos embrionarios no se observaron lesiones.

Los cultivos efectuados a partir de material del área de inoculación fueron positivos para los tres embriones, con crecimiento de escasas colonias. Los frotes positivos mostraron algunas formaciones micelianas débilmente P.A.S. positivas y pocas formas aisladas (fig. 21).

Cuando tres embriones de 17 días fueron inoculados, se obtuvo una supervivencia absoluta, no lográndose demostrar infección alguna en los animales.

La inoculación de la fase levaduriforme en cinco embriones de 12 días, produjo la muerte de todos ellos en un espacio comprendido entre las 48 y 96 horas siguientes. En el estudio microscópico notamos elementos levaduriformes, seudomicelio y bacilariformes. Algunas de estas formas se notaron débilmente P.A.S. positivas.

Al inocular tres embriones de 18 días con esta fase del hongo, obtuvimos una supervivencia completa.

En la mayoría de las piezas estudiadas, pudimos comprobar en los cortes la presencia del hongo con las características morfológicas y tintoriales descritas en los frotes. En general, se notó en la región de inoculación, una moderada infiltración celular con predominio de células rojas (figs. 22 y 23).

De tres testigos inoculados con 12 días, solamente uno murió a las 48 horas siguientes, presentando hemorragia y edema en la pata inoculada.

Inoculación en membrana de la cáscara

De seis embriones inoculados con 4 días de incubación, murieron dos de ellos a los dos y cuatro días siguientes. El estudio micológico nos demostró cómo la membrana de la cáscara estaba invadida por trozos micelianos irregulares, gran cantidad de conidias pequeñas, conidias grandes de gruesa e irre-

gular pared, blastosporas, seudomicelio pleomórfico y formas en cigarro (fig. 24). Muchos de estos elementos se mostraron débilmente positivos con el P.A.S., especialmente los micelianos (fig. 25).

Los cultivos de esta membrana fueron positivos con gran cantidad de colonias. En el embrión que murió a los cuatro días de inoculado, se comprobó la invasión de la membrana coriónica, de los anexos alantoicos y del líquido alantoico (fig. 26). Los polluelos nacidos, no manifestaron infección alguna y la mayoría de las membranas de la cáscara dieron cultivos positivos aunque no con la misma intensidad que los dos embriones que murieron.

Embriones con un período de incubación comprendido entre los 8 y 15 días no lograron infectarse. Los cultivos de la membrana de la cáscara, resultaron ser positivos con escasas colonias a los seis días. La morfología del hongo, típicamente pleomórfica, presentábase de preferencia en formas micelianas cortas e irregulares, débilmente teñidas y, en conidias pequeñas y piriformes.

Cinco testigos inoculados con solución salina estéril, de seis días, nacieron normalmente.

Inoculación endovenosa

Para realizar esta inoculación escogimos venas alantoicas por ofrecer mayores facilidades técnicas. Embriones de 13 a 16 días mueren entre las dos y doce horas siguientes. De 20 huevos, solamente en dos de ellos pudimos lograr hemocultivos positivos (monocoloniales) seis días después. En las preparaciones de sangre de otros territorios embrionarios y en los cortes efectuados, no se logró poner en evidencia elementos del hongo.

Macroscópicamente, los embriones y líquidos embrionarios se presentaron moderadamente hemorrágicos. Sin embargo puede asumirse que gran parte de la hemorragia hallada en los líquidos se debiera a sangre extravasada.

De 20 embriones inoculados con solución salina estéril con períodos de incubación entre los 13 y 16 días, ocho murieron entre las 2 y 28 horas y cuatro entre las 30 y 72 horas siguientes y el resto nacieron normalmente.

DISCUSION Y CONCLUSIONES

En las regiones de inoculación se pudo demostrar una diferente susceptibilidad a la infección, inclusive variable en algunas de ellas, según el estado de desarrollo embrionario. Con respecto a esto último, Buddingh (3) al analizar los procesos infecciosos en embriones de pollo, dice: "Las agudas o marcadas diferencias en los efectos producidos por los diversos agentes infecciosos específicos, en los diferentes estadios de desarrollo embrionario, sugieren investigaciones sobre el problema de la susceptibilidad a enfermedades infecciosas, por parte de un organismo uniforme que no está dotado de la facultad de responder con la producción de anticuerpos humorales específicos". Hamilton (4), al considerar el cambio de sensibilidad de las células a los agentes infecciosos, opina que el mismo va aparejado con la progresiva diferenciación celu-

lar, y que parece probable, por lo tanto, que este cambio sea debido a una diferenciación de las proteínas que son inapropiadas para el desarrollo de los microorganismos o que activamente los combaten formando anticuerpos específicos.

No es fácil interpretar en una forma global los resultados que se obtuvieron. Ello es comprensible, si vemos que las inoculaciones, en su mayoría, se efectuaron en un lapso variable comprendido entre los 4 y 12 días de incubación, durante el cual existen períodos en que los embriones resultan ser más susceptibles a las infecciones, los traumatismos y otros factores extrínsecos e intrínsecos (4) (10).

En saco vitelino, los resultados nos indican la existencia de un proceso de adaptación progresivo del hongo con predominio y abundancia de la fase levaduriforme en última instancia. Estos resultados son comparables a los obtenidos por BRUECK y BUDDINGH (1) cuando inocularon pus de lesiones humanas de esporotricosis en saco vitelino. Sin embargo, los autores no indican la supervivencia lograda.

En membrana corioalantoica, se observa ya a los siete días el establecimiento de la fase levaduriforme o tisular del hongo, notándose un paso progresivo bien definido a partir del inóculo micelial. SALBIN (9) al hablar del *S. schenckii* como uno de los hongos más pleomórficos, reconoció por lo menos cuatro fases de desarrollo en medios especiales in vitro: fase levaduriforme monogemante, fase levaduriforme con gemas múltiples, fase de hifas abortivas y la fase micelial. En la M.C.A. logramos observar los mismos elementos pero en un orden inverso, de tal manera que en los primeros casos se notaron elementos micelianos aún con fructificaciones, luego hifas abortivas, levaduras con gemas múltiples y por último una fase de elementos monogemantes y agemantes. El crecimiento del hongo en este anexo fue localizado y abundante, con escasa tendencia a difundirse a otras regiones. VISCO (10) al notar el mismo hecho en la inoculación de la M.C.A. con *Candida albicans,* aduce que siendo la misma un órgano mesenquimatoso, representaría un obstáculo eficaz a la difusión del hongo. En nuestro caso cabría la misma interpretación.

Las condiciones que ofrece la cavidad alantoica no son propicias para la proliferación del hongo. Estando la misma delimitada por paredes endodérmicas y, conteniendo el líquido alantoico como producto fluido en que se acumulan diversas sustancias, se presenta para el hongo un medio aparentemente desfavorable, al cual responde con predominio de formas levaduriformes. Conforme avanza el período post-inoculatorio la pobreza de los cultivos y de los elementos del hongo en los frotes estudiados, era más evidente. El comprometimiento del saco vitelino es un factor importante en el índice de mortalidad obtenido.

En la cavidad extraembrionaria, los resultados hallados indican una profusa difusión del hongo, lo cual es debido a la especial situación del inóculo. Por esta vía, el hongo encuentra un medio favorable para su prolefiración, mostrándose los embriones muy susceptibles.

Por los resultados obtenidos en los frotes y cultivos, se demuestra que

en el albumen el hongo se localiza en la región inoculada. El albumen, por sus características físicas, impide la difusión del hongo hacia otras regiones, siendo además un medio nutritivamente inadecuado como lo demuestran los resultados hallados en los cultivos y frotes. La mayoría de las formas del hongo dieron una reacción P.A.S. débil, lo cual podría interpretarse como una degeneración en este medio.

La gran susceptibilidad del embrión de pollo a la infección por la vía amniótica, se hizo patente con la muerte de todos los embriones en un período de 24 a 48 horas, lo cual explica también que no se lograra demostrar una diseminación del hongo a los tejidos intrínsecos del embrión. A pesar del corto período post-inoculatorio se observa que el medio amniótico es favorable para el desarrollo de la fase levaduriforme.

En la inoculación intracerebral se puede observar que la susceptibilidad disminuye en embriones de más de 16 días aunque el resultado siempre fue fatal. La inoculación de la fase levaduriforme prolonga un poco más el período de supervivencia.

Por la vía intramuscular la susceptibilidad disminuye con la edad del embrión, lográndose una supervivencia absoluta a los 17 días. La morfología que presenta el hongo en el material muscular, sugiere degeneración de los elementos micelianos que se muestran débilmente P.A.S. positivos. Resultados semejantes se obtuvieron con la fase levaduriforme.

Por la vía endovenosa el alto porcentaje de muertes no guarda relación con las lesiones observadas en los embriones. Por lo tanto, es difícil creer que las muertes sean debidas exclusivamente al proceso infeccioso sobre todo tomando en cuenta la mortalidad alta observada en los testigos. La aplicación de este tipo de inoculación tiene entonces un campo bastante limitado en estudios similares.

La membrana externa de la cáscara, de naturaleza queratínica, no favorece en mucho la proliferación del *S. schenckii.* Los embriones que murieron, probablemente fue debido a condiciones propias de la membrana de la cáscara. El estudio microscópico destaca el pleomorfismo del hongo con elementos débilmente P.A.S. positivos, notándose degeneración especialmente en las formas micelianas y en las conidias.

Nuestra experiencia comprueba algunos caracteres propios del *S. schenckii* como parásito y como organismo típicamente pleomórfico. Así, el hongo conforme se adapta a las diferentes regiones embrionarias, con la progresiva incubación, va adquiriendo una morfología cada vez más regular llegando a predominar en muchos casos, el aspecto levaduriforme, en seudomicelio y las formas de cigarro. El tiempo de supervivencia en algunos casos fue el motivo para que no se llevara a cabo la transformación. Muchas de las formas micelianas que se observaron, es lógico suponer que fuesen porciones del inóculo original, que por sus características daban la impresión de hallarse en vías de degeneración.

En el aspecto patogénico del *S. schenckii* para el embrión de pollo, es posible que la producción de sustancias tóxicas como resultado de la proli-

feración del hongo o de la degeneración del mismo, jueguen un papel muy importante en los resultados logrados. No debemos descartar las características individuales de los embriones y los efectos traumáticos que podrían provocar alguna contradicción en los resultados obtenidos.

RESUMEN

Se estudian los caracteres de la inoculación experimental del *S. schenckii* en el embrión de pollo, por diez vías diferentes, a saber: saco vitelino, membrana corioalantoica, cavidades alantoica, extraembrionaria y amniótica, saco del albumen, intracerebral, intramuscular, endovenosa y en la membrana de la cáscara (testácea). Para cada una de las inoculaciones se utilizó, en general, lotes de cinco huevos embrionados. De cada región inoculada y de cada embrión en particular, se hace un estudio micológico detallado y un estudio microscópico anatomopatológico, utilizando las técnicas de coloración P.A.S., Gram y hematoxilina eosina.

Se observa que los diferentes tejidos y fluidos del embrión presentan un medio nutricional y ciertas condiciones propias que hacen posible un diferente comportamiento del hongo en ellos. Se comprueba el pleomorfismo del hongo en los tejidos y líquidos embrionarios (micelio vegetativo, micelio reproductor, seudomicelio, promicelio, blastogénesis con células mono y multigemantes, conidias pequeñas y grandes, elementos redondeados y formas típicamente en naveta o cigarro), y en ningún caso se logra encontrar las formaciones asteroides descritas en otros animales de experimentación.

SUMMARY

Embryonated cricken eggs were inoculated with *Sporotrichum schenckii* by ten different routes: yolk sac, chorio-allantoic membrane, allantoic, extra-embryonary, and amniotic cavities, albumen sac, brain, muscle, blood vessels, and shell membrane. Five eggs were used in each type of inoculation. Detailed mycological studies and anatomo-pathologic microscopic examination were carried out, using P.A.S., Gram and hematoxyilin-eosin techniques.

The fungus behaved differently in the various tissues and fluids of the embryo. Pleomorphism was observed, including vegetative mycelium, reproductive mycelium, pseudomycelium, promycelium, blastospore production with one or several buds, small and large conidia, rounded and cigar-shaped cells. No asteroid formations were found.

AGRADECIMIENTO

Expreso mi reconocimiento muy sincero al Profesor Renato Soto P. por los valiosos consejos que me brindó durante la realización de este trabajo. Asimismo, agradezco profundamente al Profesor Rodrigo Zeledón su estimable ayuda en la presentación y revisión del manuscrito.

BIBLIOGRAFIA

1. Brueck, J. W., & J. Buddingh
   1951. Propagation of pathogenic fungi in the yolk sac of embryonated eggs. *Proc. Soc. Exp. Biol.*, 76: 258-261.

2. Buddingh, G. J.
   1952. Chick-embryo technics. En Rivers, T. M.: *Viral and Rickettsial infections of man.* II Ed. xvi + 719 pp. J. B. Lippincott Co., Phil.

3. Buddingh, G. J.
   1952. Bacterial and mycotic infections of the chick embryo. *Ann. N. Y. Acad. Sci.*, 55: 282-287.

4. Hamilton, H. L.
   1952. Sensitive periods during development. En Miner, R. W.: *The chick embryo in biological research. Ann. N. Y. Acad. Sci.*, 55: 37-344.

5. Langeron, M.
   1949. *Précis de microscopie.* 7 ed. viii + 1430 pp. Masson et Cie.

6. Lillie, R. D.
   1954. *Histopathologic technic, and practical histochemistry.* ix + 501 pp. Blakiston Co., Inc., N. Y.

7. Mello, R. de P., L. de P. Mello & A. Perez
   1955. Identificação de cogumelos patogenicos por meio da coloração acido periodico-Schiff. *Mem. Inst. Oswaldo Cruz,* 53: 519-524.

8. Moore, M.
   1941. The chorio-allantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi. *Amer. J. Path.*, 17: 103-125.

9. Salbin S. B.
   1947. Multiple budding in *Sporotrichum schenckii* Matruchot. *J. Invest. Dermat.*, 9: 315-320.

10. Visco, G.
    1959. Ricerche sulla candidosi sperimentale dell'embrione di pollo. 1.—Le caratteristiche generali del processo. *Riv. Ist. Sieroter. Italiano*, 24: 29-46.

Fig. 1: Aspecto del inóculo micelial utilizado en nuestras experiencias (Col. P.A.S., 1000 ×).

Fig. 2: Aspecto del inóculo levaduriforme utilizado en nuestras experiencias (Col. P.A.S., 1000 ×).

Fig. 3: Saco vitelino (embrión muerto a los 3 días): obsérvese seudomicelio y blastogénesis (Col. P.A.S., 450 ×).

Fig. 4: Saco vitelino (embrión muerto a los 4 días): nótese la presencia de blastogénesis, seudomicelio y de células multigemantes (Col. P.A.S., 1000 ×).

Fig. 5: Corte histológico de saco y contenido vitelinos (embrión muerto a los 13 días): se aprecia una profusa invasión del hongo especialmente en el área subyacente al saco vitelino (Col. P.A.S., 450 ×).

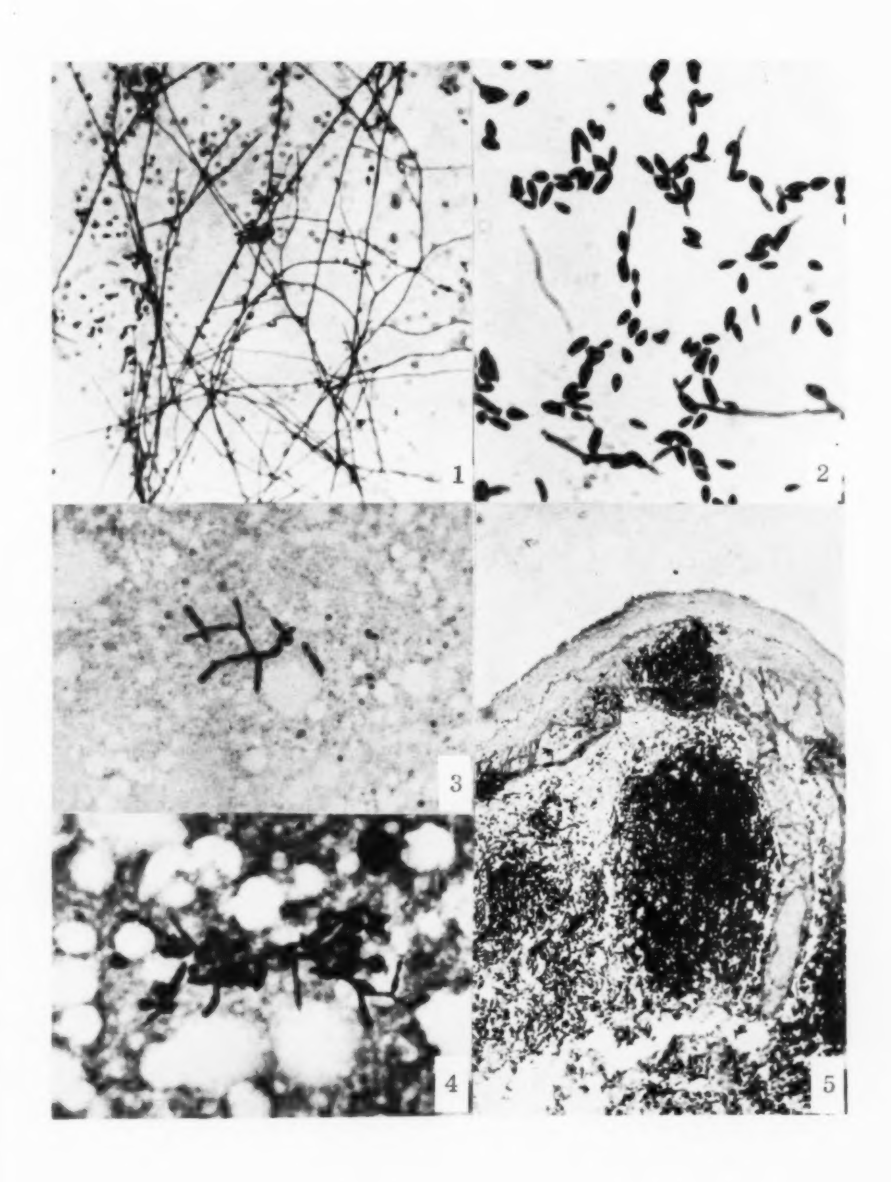

1
2
3
4
5

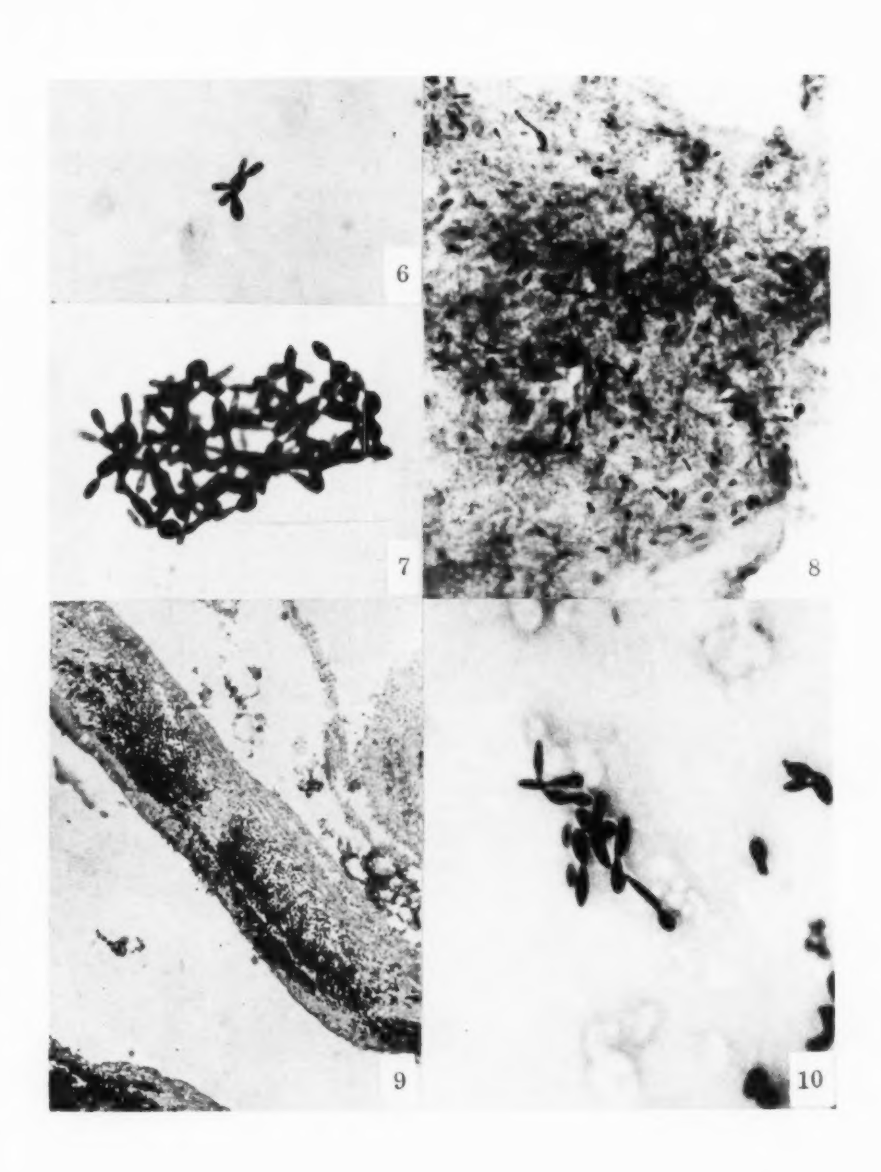
Fig. 6: Membrana corioalantoica (embrión muerto a los 4 días): se aprecia una blastospora multigemante (Col. P.A.S., 1000 ×).

Fig. 7: Membrana corioalantoica (embrión muerto a los 5 días): nótese seudomicelio de células redondeadas y alargadas, algunas multigemantes (Col. P.A.S., 1000 ×).

Fig. 8: Membrana corioalantoica (embrión muerto a los 7 días): nótese el predominio marcado de las formas en cigarro. También se observa blastogénesis (Col. P.A.S., 450 ×).

Fig. 9: Corte de membrana corioalantoica (embrión muerto a los 7 días): se aprecia una profusa invasión de la membrana, especialmente en las regiones coriónica y subcoriónica (Col. P.A.S., 450 ×).

Fig. 10: Cavidad alantoica; líquido alantoico (embrión matado intencionalmente a los 5 días): se aprecia blastogénesis, con algunas células multigemantes (Col. P.A.S., 1000 ×).



6
7
8
9
10

Fig. 11: Cavidad extraembrionaria; preparación de la pared externa del anexo alantoico (embrión muerto a los 4 días): se observa un predominio de formas promicelianas y algunos elementos aislados (Col. P.A.S., 450 ×).

Fig. 12: Secreción extraembrionaria (embrión muerto a los 6 días): obsérvese micelio vegetativo sinuoso poco teñido en algunas regiones (Col. P.A.S., 450 ×).

Fig. 13: Cavidad extraembrionaria; membrana amniótica (embrión muerto a los 4 días): obsérvese formas típicamente promicelianas (Col. P.A.S., 1000 ×).

Fig. 14: Saco del albumen (embrión matado intencionalmente a los 8 días): obsérvese el micelio irregular, pleomórfico, poco coloreado (Col. P.A.S., 450 ×).

Fig. 15: Saco del albumen (embrión muerto a los 14 días): obsérvese las típicas formas alargadas en cigarro (Col. P.A.S., 1000 ×).

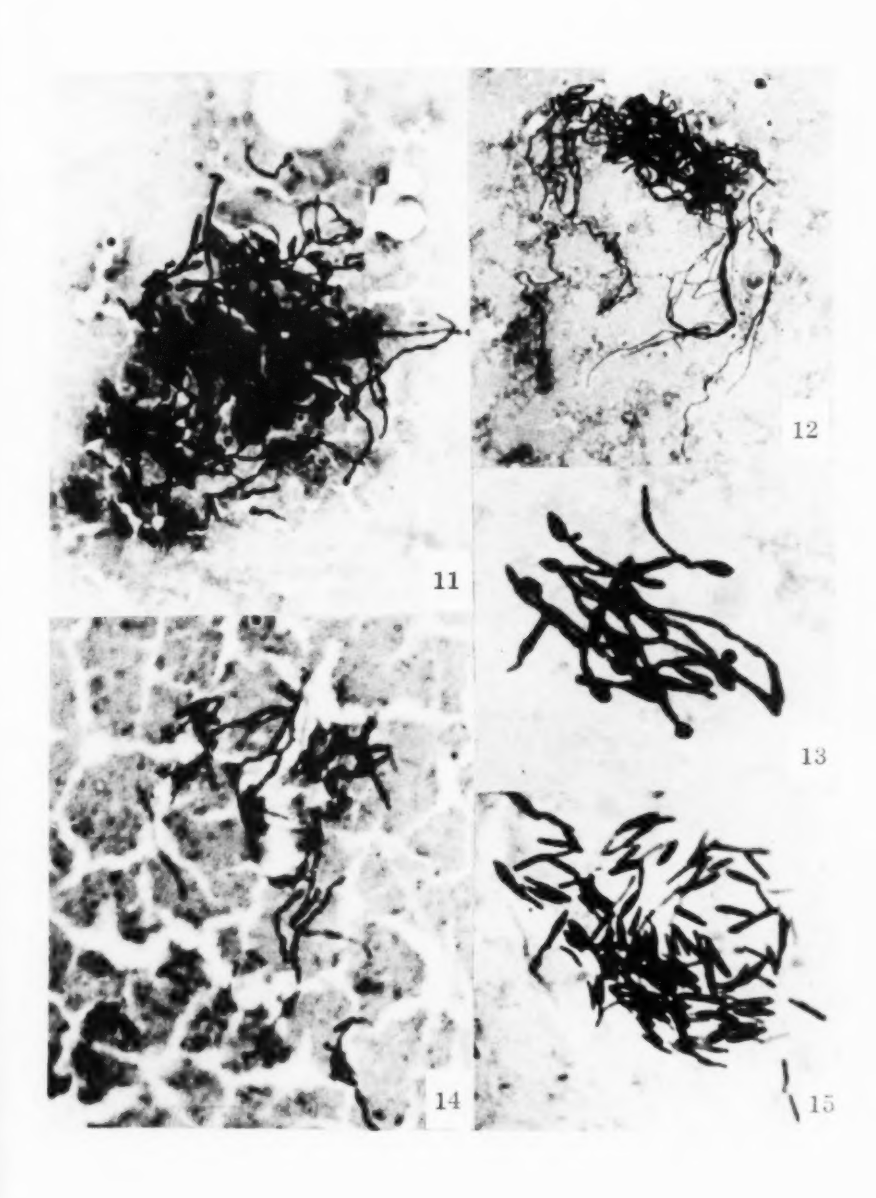

11
12
13
14
15

Fig. 16: Saco del albumen (embrión nacido a los 23 días): se aprecian elementos cocoides, unos en desintegración, otros con cortas proyecciones a manera de promicelio y algunos con su pared rota (Col. P.A.S., 1000 ×).

Fig. 17: Cavidad amniótica, membrana amniótica (embrión muerto a las 24 horas): se aprecia micelio bien desarrollado, sinuoso, predominantemente vegetativo (Col. P.A.S., 450).

Fig. 18: Cavidad amniótica, líquido amniótico (embrión muerto a las 48 horas): se aprecian seudomicelios constituídos por blastosporas redondeadas y restos micelianos poco coloreados (Col. P.A.S., 1000 ×).

Fig. 19: Cavidad amniótica, membrana amniótica (embrión muerto a las 48 horas): obsérvese hifas esporógenas con fructificaciones laterales, seudomicelio corto y blastosporas aisladas mono y digemantes (Col. P.A.S., 450 ×).

Fig. 20: Intracerebral, corte histológico (embrión muerto a las 38 horas): se observa cúmulo de elementos blastosporados dispuestos a manera de nido (Col. P.A.S., 450 ×).

Fig. 21: Intramuscular (embrión muerto a las 72 horas): se aprecia micelio irregular y poco coloreado (Col. P.A.S., 450 ×).

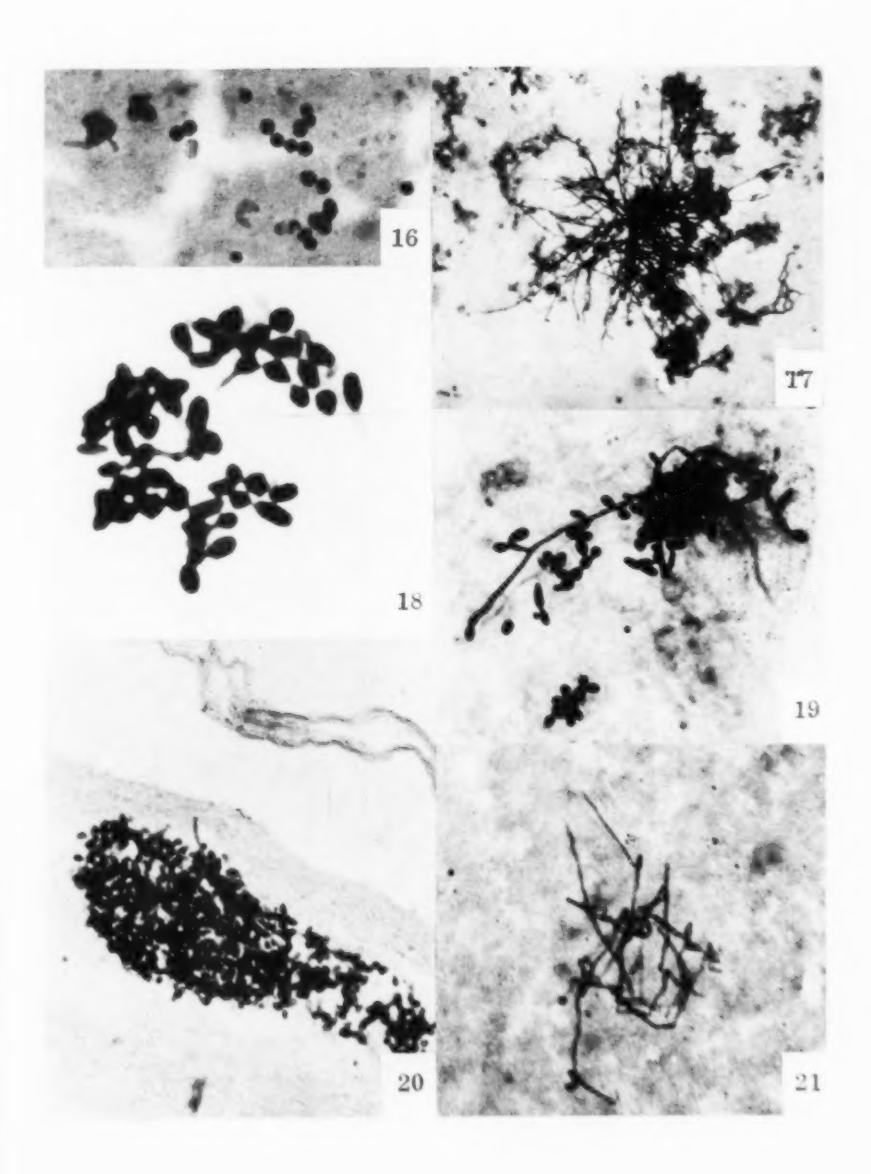

16
17
18
19
20
21

Fig. 22: Intramuscular, corte histológico (embrión muerto a las 72 horas): se observan las hifas cortadas en varias direcciones y la infiltración prevalentemente de células rojas (Col. P.A.S., 450 ×).

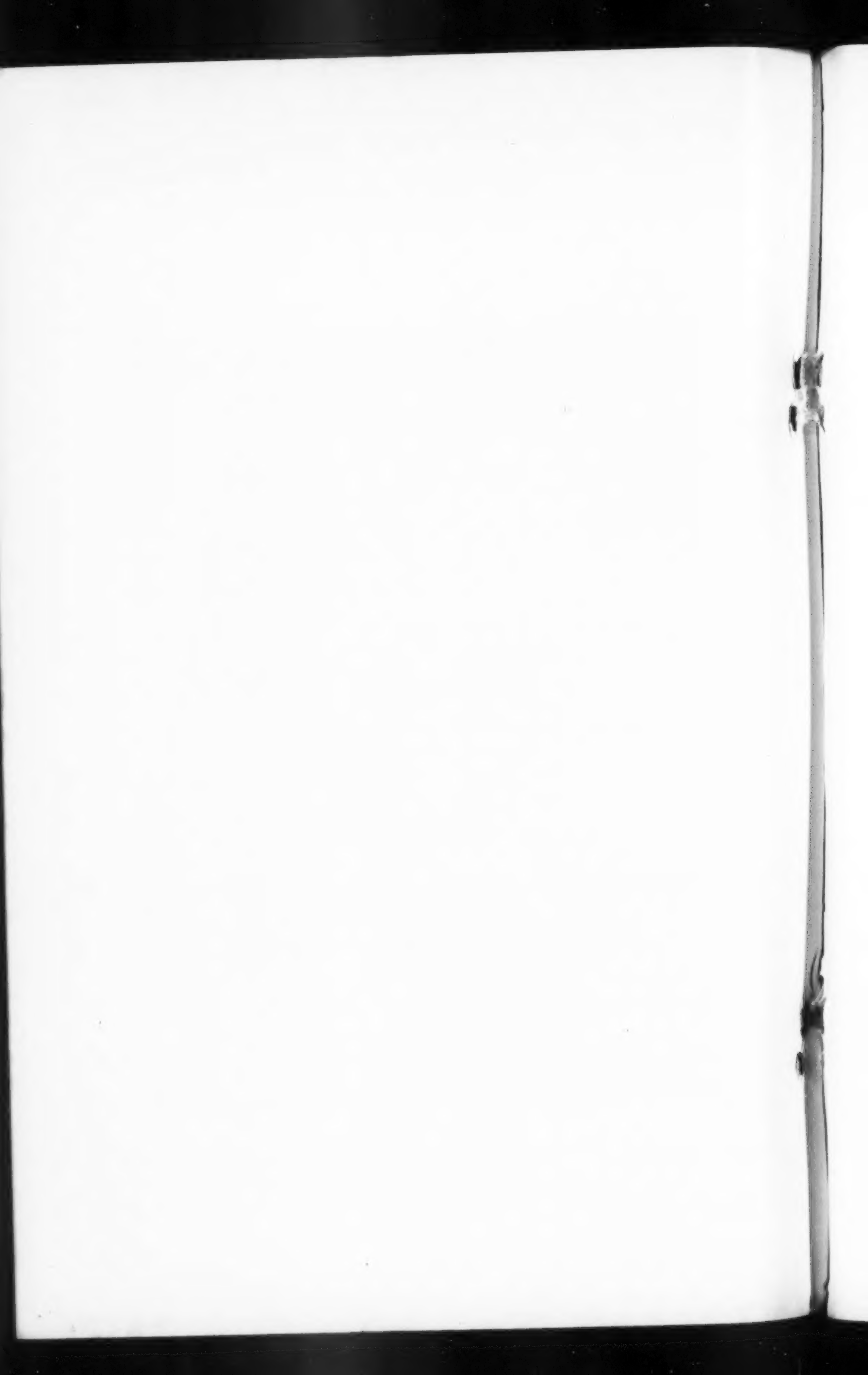
Fig. 23: Intramuscular, corte histológico (embrión muerto a las 48 horas): se observan los elementos blastosporados entre las fibras musculares (Col. P.A.S., 450 ×).

Fig. 24: Membrana de la cáscara (preparación hecha a los 7 días después de inoculado un embrión de 10 días): se nota el predominio de formas redondeadas pequeñas y grandes, algunas poco coloreadas y, algunos restos micelianos también poco teñidos (Col. P.A.S., 450 ×).

Fig. 25: Membrana de la cáscara (de igual procedencia que la anterior): nótese el micelio poco teñido e irregular y las escasas formas aisladas (Col. P.A.S., 450 ×)

Fig. 26: Membrana de la cáscara, corte histológico (embrión de 4 días muerto a las 48 horas): obsérvese sobre la membrana de la cáscara la gran cantidad de los elementos del hongo, con prevalencia de trozos micelianos irregulares y formas aisladas (Col. P.A.S., 450 ×).


22
24
25
26



Rev. Biol. Trop., 8 (1): 145-146, 1960

Intradermo-reacción de Montenegro con antígeno de Strigomonas oncopelti. Nota Previa.

por

Rodrigo Zeledón*

Walter Hidalgo R.**

Hilda X. de Hidalgo**

(Recibido para su publicación el 21 de junio de 1960)

La presente nota tiene por objeto dar a conocer los resultados obtenidos en intradermo-reacciones de Montenegro, con un antígeno heterólogo, practicadas en pacientes internados en el Hospital San Juan de Dios de San José. El antígeno preparado con *Strigomonas oncopelti*, un flagelado cultivable en medios bastante más simples que los requeridos por *Leishmania brasiliensis*, fue usado en las intradermo-reacciones al lado del antígeno específico. *S. oncopelti* se cultivó en un medio líquido, autoclavable, libre de proteínas (cf. Zeledón, R., *J. Parasit.*, 45: 652, 1959) y *L. brasiliensis* en medio de N.N.N. a temperatura ambiente.

Los flagelados fueron cosechados a los 6 y 9 días respectivamente, lavados por centrifugación tres veces con solución salina al 0,9 por ciento y resuspendidos en la misma solución con mertiolato al 1/5000. Las concentraciones finales fueron de 7 millones de leptómonas por mililitro para *S. oncopelti* y de 2.5 millones para *L. brasiliensis*. Antes de ser usados, los antígenos fueron sometidos a pruebas bacteriológicas de esterilidad y a congelación y descongelación por tres veces consecutivas.

Cada paciente recibió en el antebrazo, 0,1 ml de cada uno de los antígenos más un control de solución salina con mertiolato a la concentración citada. Del grupo de enfermos, 26 en total, 19 reaccionaron negativamente y 7 mostraron reacción positiva a ambos antígenos. De estos 7 pacientes, todos con

* Departamento de Parasitología, Facultad de Microbiología, Universidad de Costa Rica.
** Laboratorio Bacteriológico, Hospital San Juan de Dios.

IMPPESA EN LOS TALLERES TIPOGRAFICOS FALCO LTDA.

